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(54) Title: MODIFIED CEA AND USES THEREOF

(57) Abstract: The invention discloses immunogenic CEA agonist polypeptides/proteins comprising a modified epitope containing the amino acid sequence YLSGADLNL, nucleic acids coding therefor, vectors and/or cells comprising said nucleic acids, and mixtures and/or compositions thereof. Methods for eliciting or inducing CEA-specific immune responses utilizing the aforementioned agents are also disclosed.

Modified CEA and Uses Thereof

FIELD OF THE INVENTION

The present invention relates to immunology, in particular to novel biologically active modified CEA agonist polypeptides/proteins containing a modified epitope therein, nucleic acids coding therefor, vectors and/or cells comprising said nucleic acid, mixtures and/or compositions of the aforementioned, and their use as immunogenic agents and/or in treatments of cancer.

BACKGROUND OF THE INVENTION

Throughout this application, various references are cited in parentheses to describe more fully the state of the art to which this invention pertains. The disclosure of these references are hereby incorporated by reference into the present disclosure.

The prospects of cancer immunotherapy rely upon the identification of tumor associated antigens which can be recognized by the immune system. Specifically, target antigens eliciting T cell-mediated responses are of critical interest. This comes from evidence that cytotoxic T lymphocytes (CILs) can induce tumor regression both in animal models (Kast W. et al (1989) Cell 59:6035; Greendberg P. *(1991)Adv. Immunol.* 49:281) and in humans (Boon T. et al. (1994) *Annu. Rev. Immunol* 12:337).

Human carcinoembryonic antigen (CEA) is a 180 kD glycoprotein expressed on the majority of colon, rectal, stomach and pancreatic tumors (Muaro et al. (1985) *Cancer* Res. 45:5769), some 50% of breast carcinomas (Steward et al. (1974) Cancer 33:1246) and 70% of lung carcinomas (Vincent, R.G. and Chu, T.M. (1978) *J. Thor. Cardiovas.* Surg. 66:320). CEA is also expressed in fetal gut tissue and to a lesser extent on normal colon epithelium. The immunogenicity of CEA has been ambiguous, with several studies reporting the presence of anti-CEA antibodies in patients (Gold et al. (1973) *Nature New Biology* 239:60; Pompecki, R. (1980) *Eur. J. Cancer* 16:973; Ura et al. (1985) *Cancer Lett.25:283;* Fuchs et al. (1988) *Cancer Immunol. Immunother.* 26:180) while other studies have not (LoGerfo et al. (1972) *Int. J Cancer* 9:344; MacSween, J.M. (1975) *Int J. Cancer* 15:246; Chester K.A. and Begent, H.J. (1984) *Clin. Exp. Immunol* 58:685). CEA was first described as a

cancer specific fetal antigen in adenocarcinoma of the human digestive tract in 1965 (Gold, P. and Freeman, 5.0. (1965) *Exp. Med.* 121:439). Since that time, CEA has been characterized as a cell surface antigen produced in excess in nearly all solid tumors of the human gastrointestinal tract. The gene for the human CEA protein has been cloned (Oikawa et al (1987) *Biochim. Biophys. Res.* 142:511-518; European Application No. EP 0346710).

Notwithstanding the aforementioned ambiguous demonstration of CEA's immunogenicity, a phase I clinical trail using a vaccinia-CEA vaccine ("rV-CEA") did demonstrate that CEA-specific cytolytic T-lymphocyte (CTL) response could be elicited in humans (Tsang, K.Y. et al. (1995) *J. Natl. Cancer Instit* 87:982-990; Tsang K.Y. et al. (1997) *Clin. Cancer Res.* 3:2439-2449). As a consequence of the studies, a CEA immunodominant CTL epitope was identified. This 9-mer (i.e. YLSGANLNL) was shown to bind to a HLA-A2 class I molecule and has been designated carcinoembryonic antigen peptide-1 ("CAP-1"). Several subsequent studies have also demonstrated the ability of the CAP-I epitope/peptide *per se* to elicit CEA-specific human CTL responses (Alters, S.E. et al. (1998) *J. Immunother.* 21:17-26; Tsang, K.Y. et al. (1997) *Supra;* Zaremba, S. et al. (1997) *Cancer Res.* 57:4570-4577). Moreover, stable CTL lines derived by culture of peripheral blood mononuclear cells (PBMCs) from rV-CEA vaccinated patients with CAP1 and interleukin (IL)-2 have recently been described (Tsang, K.Y. (1997) *Supra).*

It is an accepted principle that when an immunogenic peptide is modified in a conserved manner (i.e. a hydrophobic amino acid is substituted with a hydrophobic amino acid) the modified peptide is likely to have similar immunogenic activity based upon the maintenance of the molecule's shape, charge and hydrophobic character. More specifically, a study by Madden (Madden et al. (1993) *Cell* 75:693) has identified specific amino acid preferences in peptides for MHC-complexing, a precursor step to T cell recognition. Madden as well as other investigators (Rammensee et al., (1995) *Immunogenetics* 41:178) have suggested that specific amino acid positions in peptides are available for T cell recognition.

Skipper et al. ((1996) *J. Exp, Med.* 183: 527) described the identification and characterization of a naturally-occurring peptide epitope of tyrosinase wherein the peptide sequence differed from that which is predicted from the DNA. This modified peptide was recognized by tyrosinase-specific human cytotoxic T-lymphocytes ("CTL") more effectively than the direct translation product, and was the only one of the two peptides to be presented by HLA-A2.1 molecules on the cell surface. The modification was a substitution of an asparagine with an aspartic acid. The authors proposed that the asparagine was N-glycosylated in the endoplasmic reticulum during protein synthesis and subsequently deamidated post-translationally.

With respect to the CEA epitope CAP1, the primary and secondary anchors positions for HLA binding at positions 2, 9, and 1 (of the epitope) are already occupied by preferred amino acids. As such, Schlom and colleagues attempted to increase CAP1 immunogenicity via the generation of epitope analogs containing single amino acid substitutions to residues predicted to interact with the T cell receptor ("TCR") of CAP1-specific CTL. One such analog epitope (YLSGADLNL; designated CAP-1-6D) was identified which demonstrated an increased immunogenicity to that of the natural epitope, but not a concomitant increase in MHC binding *per se* (i.e. it behaved as an agonist; Zaremba, S. et al (1997) *Supra).*

The present invention discloses novel modified CEA agonist polypeptides/proteins containing a modified epitope therein, nucleic acids coding therefor, vectors comprising said nucleic acids, mixtures and/or compositions of the aforementioned agents, and their advantageous use in generating CEA-specific immune responses and/or in the treatment of cancers.

SUMMARY OF THE INVENTION

The present invention encompasses novel modified CEA agonist polypeptides/proteins comprising a modified epitope containing the sequence YLSGADLNL, nucleic acids coding therefore, vectors (such as recombinant virus and/or bacteria) and/or cells (such as antigen-presenting cells) comprising said nucleic acids, and mixtures and/or compositions of the aforementioned. All of these

aforementioned agents, mixtures and compositions are characterized by their ability to induce or elicit an immune response against a CEA protein or fragment thereof, a CEA agonist polypeptide containing said modified epitope, a normal or modified CEA epitope, or cells binding or expressing the aforementioned CEA protein/fragment, CEA agonist polypeptide, or normal/modified CEA epitope.

Accordingly, in one embodiment of the invention a CEA agonist polypeptide/protein is provided comprising a modified epitope of CEA, wherein said modified epitope contains the sequence YLSGADLNL.

In a further embodiment of the invention, the CEA agonist polypeptide/protein has the amino acid sequence of SEQ ID NO: 1 (Figure 1).

As previously noted, embodiments of the invention encompass nucleic acids coding for the aforementioned CEA agonist polypeptides/proteins. Accordingly, embodiments of the invention consist/comprise the nucleic acid sequence of SEQ ID NO: 2 (Figure 1). In further embodiments of the invention, the nucleic acid is a DNA selected from the group consisting of viral nucleic acid, plasmid, bacterial DNA, naked/free DNA, and RNA. In yet further embodiments, the viral nucleic acid is selected from the group consisting of adenovirus, alphavirus and poxvirus. In still yet further embodiments, the poxvirus is selected from the group consisting of avipox, suipox and orthopox. In still yet further embodiments, the poxviral nucleic acid is selected from the group consisting of TROVAC, NYVAC, ALVAC, MVA, Adeno-Associated Virus (AAV), Wyeth; and PoxvacTC.

Additional embodiments of the invention are contemplated encompassing nucleic acids comprising a sequence encoding for the CEA agonist polypeptide/protein in addition to a second sequence encoding at least one member selected from the group comprising cytokines, lymphokines, and co-stimulatory molecules.

Embodiments of the invention further contemplate vectors comprising the

nucleic acid(s) of the invention. In particular embodiments, these vectors may be either recombinant viruses or bacteria. In further embodiments, the recombinant viruses are selected from the group consisting of adenovirus, alphavirus and poxvirus. In yet further embodiments, the poxvirus is selected for the group consisting of avipox, orthopox and suipox; particular embodiments encompass ALVAC, NYVAC, TROVAC, MVA, Wyeth and Poxvac-TC.

The invention further provides for cells comprising the aforementioned nucleic acid(s) of the invention, wherein said cells express the CEA agonist polypeptide/protein of the invention. In further embodiments, the cells expressing the CEA agonist polypeptide/protein also express a MHC HLA class 1 molecule. In yet further embodiments, the cells expressing the polypeptide are antigen-presenting cells.

Embodiments of the invention further encompass mixtures and/or compositions of the aforementioned CEA agonist polypeptides/proteins, nucleic acids, vectors, and cells. These mixtures and/or compositions, may optionally include adjuvants.

The invention further provides a method of inducing an immune response in an animal directed against:

(i) a CEA protein or fragment thereof; and/or

(ii) a CEA agonist polypeptide/protein of the invention; and/or

(iii) a CEA epitope; and/or

(iv) a modified CEA epitope; and/or

(v) cells expressing a CEA protein or fragment thereof, CEA agonist polypeptide/protein of the invention, CEA epitope, modified CEA

epitope; and/or;

(vi) cells binding a CEA protein or fragment thereof, CEA agonist polypeptide/protein of the invention, CEA epitope, modified CEA epitope,

comprising administering to said animal a CEA agonist polypeptide/protein, nucleic acid, vector, cell, or mixture and/or composition of the invention in an amount sufficient to induce an immune response.

The invention further contemplates a method of inhibiting a CEA epitope expressing carcinoma cell in a patient comprising administering to said patient an effective amount of a CEA agonist polypeptide/protein, nucleic acid, vector, cell, or mixture and/or composition of the invention.

The invention in yet a further aspect provides for a treatment for cancer comprising any one of the aforementioned methods for inducing immune responses and/or inhibiting carcinoma cells expressing a CEA epitope.

Other features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating particular embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed, description.

BRIEF DESCRIPTION OF THE FIGURES

The present invention will be further understood from the following description with reference to the figures, in which:

Figure 1 depicts the nucleic acid and amino acid sequence of an embodiment of the invention encompassing modified CEA.

Figure 2 depicts 3 schematic representations encompassing the genomic structure of particular recombinant fowlpox, vaccinia and MVA

constructs expressing modified CEA.

Figure 3 depicts a schematic representation of the *XhoI* restriction map profile of an ALVAC(2)-CEA (modified)/human B7.1 construct.

Figure 4 depicts the nucleic acid sequence of the H6-promoted CEA (modified)/human B7.1 insertion cassette used in the production of the construct of Figure 3.

Figure 5 depicts the results of an immunoprecipitation analysis of HeLa cells infected with various ALVAC recombinant constructs.

Figure 6 depicts the results of a western blot analysis of HeLa cells infected with various ALVAC recombinant constructs.

DETAILED DESCRIPTION OF THE INVENTION

The invention discloses CEA agonist polypeptides/proteins comprising a modified CEA epitope wherein said modified CEA epitope comprises the sequence YLSGADLNL, nucleic acids coding therefor, vectors and/or cells comprising said nucleic acids (collectively designated as "agents" of the invention), and mixtures/compositions of the aforementioned. All of the aforementioned agents and mixtures/compositions of the invention have the ability to induce or elicit an immune response against a CEA protein fragment thereof, a CEA agonist polypeptide/protein of the invention, a CEA epitope and/or modified CEA epitope, and/or cells binding or expressing the aforementioned. An "immune response" is defined as any response of the immune system, for example, of either a cell-mediated (i.e. cytotoxic T-lymphocyte mediated) or humoral (i.e. antibody mediated) nature. As is known to those skilled in the art, various assays/methodologies exist for the assessment and/or monitoring of immunological responses.

Within the context of cell-mediated immune responses, tumor associated antigen proteins (such as CEA) are processed by intracellular proteases into smaller epitope peptides which are subsequently transported to the cell surface tightly bound in a cleft on an MHC HLA class I molecule. T cells recognize these small epitope peptides only when presented in association with MHC HLA Class I molecules on

the surface of appropriate cells. Analogously, in the context of humoral immune responses proteins can be processed into smaller epitope peptides which are subsequently presented on cell surfaces (i.e. antigen presenting cells) in association with MHC HLA class II molecules. Said complexes are recognized by appropriate cells of the humoral immune system.

As is well known to those skilled in the art, short peptides (i.e. epitopes) composed of amino acid sequences of about 8 to 12 amino acids derived from antigens are capable of binding directly within the cleft of an HLA class I molecule without intracellular processing. As previously noted, a number of such epitope peptides derived from CEA have been identified. Moreover, some of these CEA-specific antigen epitopes have demonstrated the capacity to induce/elicit immune responses wherein appropriate CEA expressing target cells are lysed. The CEA agonist polypeptides/proteins of the present invention (comprising a modified CEA epitope) elicit an improved immune' response (hence, deemed to be CEA "agonist(s)") by comparison to that observed when normal/unmodified CEA (comprising normal epitopes) is employed as an immunogen.

As encompassed by this invention, the CEA agonist polypeptides/proteins comprise a modified epitope containing the amino acid sequence YLSGADLNL (designated "CAP16D"). The counterpart sequence of the naturally occurring epitope in unmodified CEA is YLSGANLNL (designated "CAP1"; Zaremba, S. et al. (1997) Cancer Res. 57:4570). In a particular embodiment of the invention, the CEA agonist polypeptide/protein has the amino acid sequence of SEQ ID NO: 1 (Figure 1). It is further recognized that CEA agonist polypeptide(s)/protein(s) embodiments of the invention encompass both precursor and mature forms of polypeptide/protein (for example, see Oikawa, S. et al. (1987) *Biochem. Biophys. Res. Commun.* 142:51 1-518).

The CEA agonist polypeptides/proteins of the invention may be prepared using a variety of methods known to one skilled in the art. Accordingly, recombinant DNA methods known to those skilled in the art can be utilized to provide these

polypeptides. Nucleic acid sequences which encode for the CEA agonist polypeptides/proteins of the invention may be incorporated in a known manner into appropriate expression vectors (i.e. recombinant expression vectors). Possible expression vectors include (but are not limited to) cosmids, plasmids, or modified viruses (e.g. replication defective retroviruses, adenoviruses and adeno-associated viruses, lentiviruses, herpes viruses, poxviruses), so long as the vector is compatible with the host cell used. The expression "vector is compatible with the host cell" is defined as contemplating that the expression vector(s) contain a nucleic acid molecule of the invention (hereinafter described) and attendant regulatory sequence(s) selected on the basis of the host cell(s) to be used for expression, said regulatory sequence(s) being operatively linked to the nucleic acid molecule. "Operatively linked" is intended to mean that the nucleic acid is linked to regulatory sequence(s) in a manner which allows expression of the nucleic acid. Suitable regulatory sequences may be derived from a variety of sources, including bacteria), fungal, or viral genes. (Forexample, see the regulatory sequences described in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). Selection of appropriate regulatory sequence(s) is dependent on the host cell(s) chosen, and may be readily accomplished by one of ordinary skill in the art. Examples of such regulatory sequences include the following: a transcriptional promoter and enhancer, RNA polymerase binding sequence, or a ribosomal binding sequence (including a translation initiation signal). Depending on the host cell chosen and the expression vector employed, other additional sequences (such as an origin of replication, additional DNA restriction sites, enhancers, and sequences confeffing inducibility of transcription) may be incorporated into the expression vector.

The aforementioned expression vectors of the invention may also contain a selectable marker gene which facilitates the selection of host cells transformed or transfected with a recombinant molecule of the invention. Examples of selectable marker genes are genes encoding a protein such as G418 and hygromycin (which confer resistance to certain drugs), β-galactosidase, chloramphenicol acetyltransferase, or firefly luciferase.

Transcription of the selectable marker gene is monitored by changes in the concentration of the selectable marker protein such as β-galactosidase, chloramphenicol acetyltransferase, or firefly luciferase. Transformant cells can be selected with appropriate selection molecules if the selectable marker gene encodes a protein conferring antibiotic resistance (i.e. G418 in context of neomycin resistance). As is known to one skilled in the art, cells that have incorporated the selectable marker gene will survive, while cells which do not have any such incorporated detectable marker will die. This makes it possible to visualize and assay for expression from recombinant expression vectors of the invention. It will also be appreciated that selectable markers can be introduced on a separate vector from the nucleic acid of interest.

The recombinant expression vectors may also contain genes which encode a fusion moiety which provides increased expression of the polypeptides of the invention; increased solubility of the polypeptides of the invention; and/or aids in the purification of a target recombinant protein by acting as a ligand in affinity purification. For example, a proteolytic cleavage site may be added to the target recombinant polypeptide to allow separation of the recombinant polypeptide peptide(s) from the fusion moiety subsequent to purification of the fusion protein.

The CEA agonist polypeptides/proteins of the invention may also be prepared by chemical synthesis using techniques well known in the chemistry of proteins such as solid phase synthesis (Merrifield (1964) *J. Am. Chem. Assoc.* 65:2149) or synthesis in homogenous solution (Methods of Organic Chemistry, E. Wansch (Ed.) Vol. 15, pts. I and II, Thieme, Stuttgart (1987)).

Additional embodiments of the invention encompass nucleic acids coding for the CEA agonist polypeptides/proteins hereinbefore described. As defined herein, "nucleic acid(s)" encompass (but is not limited to) viral nucleic acid(s), plasmid(s), bacterial DNA, naked/free DNA and RNA. The nucleic acids encompass both single and double stranded forms. As such, these nucleic acids comprise the relevant base

sequences coding for the aforementioned polypeptides. For purposes of definitiveness, the "relevant base sequence's coding for the aforementioned polypeptides" further encompass complementary nucleic acid sequences.

In one embodiment of the invention, the nucleic acid has the sequence denoted by SEQ ID NO:2 (Figure 1). In further embodiments of the invention, the nucleic acid comprises this sequence (i.e. SEQ ID NO:2 (Figure 1)). It is further recognized that additional embodiments of the invention may consist of and/or comprise nucleic acid sequences coding for precursor or mature CEA agonist polypeptide(s)/protein(s) (for example, see Oikawa, S. et al. (1987) *Supra).*

Bacterial DNA useful in embodiments of the invention are known to those of ordinary skill in the art. These bacteria include, for example, *Shigella, Salmonella, Vibrio cholerae, Lactobacillus, Bacille Calmette Guérin (BCG)*, and *Streptococcus.* In bacterial DNA embodiments of the invention, nucleic acid of the invention may be inserted into the bacterial génome, can remain in a free state, or be parried on a plasmid.

Viral nucleic acid embodiments of the invention may be derived from a poxvirus or other virus such as adenovirus or alphavirus. Preferably the viral nucleic acid is incapable of integration in recipient animal cells. The elements for expression from said nucleic acid may include a promoter suitable for expression in recipient animal cells.

Embodiments of the invention encompass poxviral nucleic acid selected from the group consisting of avipox, orthopox, and suipox nucleic acid. Particular embodiments encompass poxviral nucleic acid selected from vaccinia, fowlpox, canary pox and swinepox; specific examples include TROVAC, NYVAC, ALVAC, MVA, Wyeth and Poxvac-TC (described in more detail below).

It is further contemplated that nucleic acids of this invention may further comprise nucleic acid sequences, encoding at least one member chosen from the

group consisting cytokines, lymphokines, and co-stimulatory molecules. Examples include (but are hot limited to) interleukin 2, interleukin 12, interleukin 6, interferon gamma, tumor necrosis factor Alpha, GM-CSF, B7.1, B7.2, ICAM-1, LFA-3, and Cd72.

Standard techniques of molecular biology for preparing and purifying nucleic acids well known to those skilled in the art can be used in the preparation of aspects of the invention (for example, as taught in *Current Protocols in Molecular Biology,* F.M. Ausubel et al. (Eds.), John Wiley and Sons, Inc, N.Y., U.S.A. (1998), Chpts. 1, 2 and 4; *Molecular Cloning: A Laboratory Manual (2nd Ed.),* J. Sambrook, E.F. Fritsch and T. Maniatis (Eds.), Cold Spring Harbor Laboratory Press, N.Y., U.S.A. (1989), Chpts. 1, 2, 3 and 7).

Aspects of this invention further encompass vector comprising, the aforementioned nucleic acids. In certain embodiments, said vectors may be recombinant viruses or bacteria.

Adenovirus vectors and methods, for their construction have been described (e.g. U.S. Patent Nos. 5994132, 5932210, 6057158 and Published PCT Applications WO 9817783, WO 9744475, WO 9961034, WO 9950292, WO 9927101, WO 9720575, WO 9640955, WO 9639534, all of which are herein incorporated by reference). Alphavirus vectors have also been described in the art and can be used in embodiments of this invention (e.g. U.S. Patent Nos. 5792462, 5739026, 5843723, 5789245, and Published PCT Applications WO 9210578, WO 9527044, WO 9531565, WO 9815636 all of which are herein incorporated by reference), as have lentivirus vectors (e.g. U.S. Patent Nos. 6013516, 5994136 and Published PCT Applications WO 9617816, WO 9712622, WO 9817815, WO 9839463, WO 9846083, WO 9915641, WO 9919501, WO 9930742, WO 9931251, WO 9851810, WO 0000600 all of which are herein incorporated by reference). Poxvirus vectors that can be used include, for example, avipox, orthopox or suipox poxvirus (as described in U.S. Patent Nos. 5364773, 4603112, 5762938, 5378457, 5494807, 5505941, 5756103, 5833975 and 5990091 all of which are herein incorporated by

reference). Poxvirus vectors comprising a nucleic acid coding for a CEA agonist polypeptide/protein of the invention can be obtained by homologous recombination as is known to one skilled in the art, as such, the nucleic acid coding for the CEA agonist polypeptide/protein is inserted into the viral genome under appropriate conditions for expression in mammalian cells (as described below).

In one embodiment of the invention the poxvirus vector is ALVAC (1) or ALVAC (2) (both of which have been derived from canarypox virus). ALVAC (1) (or ALVAC (2)) does not productively replicate in non-avian hosts, a characteristic thought to improve its safety profile. ALVAC (1) is an attenuated canarypox virus-based vector that was a plaque-cloned derivative of the licensed canarypox vaccine, Kanapox (Tartaglia et al. (1992) *Virology* 188:217; U.S. Patent Nos. 5505941, 5756103 and 5833975 all of which are incorporated herein by reference). ALVAC (1) has some general properties which are the same as some general properties of Kanapox. ALVAC-based recombinant viruses expressing extrinsic immunogens have also been demonstrated efficacious as vaccine vectors (Tartaglla et al, In AIDS Research Reviews (vol. 3) Koff W., Wong-Staol F. and Kenedy R.C. (eds.), Marcel Dekker NY, pp. 361-378 (1993a); Tartaglia, J. et al. (1993b) *J. Vivol.* 67:2370). For instance, mice immunized with an ALVAC (1) recombinant expressing the rabies virus glypoprotein were protected from lethal challenge with rabies virus (Tartaglia, J. et at, (1992) *supra)* demonstrating the potential for ALVAC (1) as a vaccine vector. ALVAC-based recombinants have also proven efficacious in dogs challenged with canine distemper virus (Taylor, J. et al. (1992) *Virology* 187:321) and rabies virus (Perkus, M.E. et al., In Combined Vaccines and Simultaneous Administration: Current Issues and Perspective, Annals of the New York Academy of Sciences (1994)), in cats challenged with feline leukemia virus (Tartaglia, J. et al., (1993b) *supra),* and in horses challenged with equine influenza virus (Taylor, J. et aI., In Proceedings of the Third International Symposium on Avian Influenza, Univ. of Wisconsin-Madison, Madison, Wisconsin, pp. 331-335 (1993)).

ALVAC (2) is a second-generation ALVAC vector in which vaccinia transcription elements E3L and K3L have been inserted within the C6 locus (U.S.

Patent No. 5990091, incorporated herein by reference). The E3L encodes a protein capable of specifically binding to dsRNA. The K3L ORF has significant homology to E1 F-2. Within ALVAC (2) the E3L gene is under the transcriptional control of its natural promoter, whereas K3L has been placed under the control of the early/late vaccine H6 promoter. The E3L and K3L genes act to inhibit PKR activity in cells infected with ALVAC (2), allowing enhancement of the level and persistence of foreign gene expression.

Additional viral vectors encompass natural host-restricted poxviruses. Fowlpox virus (FPV) is the prototypic virus of the Avipox genus of the Poxvirus family. Replication of avipox viruses is limited to avian species (Matthews, R.E.F. (1982) *Intervirology* 17:42) and there are no reports in the literature of avipox virus causing a productive infection in any non-avian species including man. This host restriction provides an inherent safety barrier to transmission of the virus to other species and makes use of avipox virus based vectors in veterinary and human applications an attractive proposition.

FPV has been used advantageously as a vector expressing immunogens from poultry pathogens. The hemagglutinin protein of a virulent avian influenza virus was expressed in an FPV recombinant. After inoculation of the recombinant into chickens and turkeys, an immune response was induced which was protective against either a homologous or a heterologous virulent influenza virus challenge (Taylor, J. et al. (1968) Vaccine 6: 504). FPV recombinants expressing the surface glycoproteins of Newcastle Disease Virus have also been developed (Taylor, J. et al. (1990) *J. Virol.* 64:1441; Edbauer, C. et al. (1990) Virology 179:901); U.S. Patent No. 5766599 incorporated herein by reference).

A highly attenuated strain of vaccinia, designated MVA, has also been used as a vector for poxvirus-based vaccines. Use of MVA is described in U.S. Patent No. 5,185,146.

Other attenuated poxvirus vectors have been prepared via genetic modification to wild type strains of vaccinia. The NYVAC vector, for example, is derived by deletion of specific virulence and host-range genes from the Copenhagen strain of vaccinia (Tartaglia, J. et al. (1992), *supra;* U.S. Patent Nos. 5364773 and 5494807 incorporated herein by reference) and has proven useful as a recombinant vector in eliciting a protective immune response against expressed foreign antigens.

Recombinant viruses can be constructed by processes known to those skilled in the art (for example, as previously described for vaccinia and avipox viruses; U.S. Patent Nos. 4769330; 4722648; 4603112; 5110587; and 5174993-all of which are incorporated herein by reference).

In further embodiments of the invention, live and/or attenuated bacteria may also be used as vectors. For example, non-toxicogenic *Vibrio cholerae* mutant strains may be useful as bacterial vectors in embodiments of this invention; as described in US Patent, No. 4,882,278 (disclosing a strain in which a substantial amount of the coding sequence of each of the two ctxA alleles has been deleted so that no functional cholera toxin is produced), WO 92111354 (strain in which the irgA locus is inactivated by mutation; this mutation can be combined in a single strain with ctxA mutations), and WO 94/1533 (deletion mutant lacking functional ctxA and attRS1 DNA sequences). These strains can be genetically engineered to express heterologous antigens, as described in WO 94/19482. (All of the aforementioned issued patent/patent applications are incorporated herein by reference). Attenuated *Salmonella typhimurium* strains, genetically engineered for recombinant expression of heterologous antigens and their use as oral immunogens are described, for example, in WO 92/11361.

As noted, those skilled in the art will readily recognize that other bacterial strains useful as bacterial vectors in embodiments of this invention include (but are not limited to) *Shigella flexneri, Streptococcus gordonii,* and *Bacille Calmette Guerin* (as described in WO 88/6626, WO 90/0594, WO 91/13157, WO 92/1796, and WO 92/21376; all of which are incorporated herein by reference). In bacterial

vector embodiments of this invention, a nucleic acid coding for a CEA agonist polypeptide/protein may be inserted into the bacterial genome, can remain in a free state, or be carried on a plasmid.

It is further contemplated that the invention encompasses vectors which comprise nucleic acids coding for at least one member from the group consisting of cytokines, lymphokines and immunostimulatory molecules. Said nucleic acid sequences can be contiguous with sequences coding for CEA agonist polypeptide/proteins, or encoded on distinct nucleic acids.

Cells comprising the aforementioned nucleic acids coding for CEA agonist polypeptides/proteins encompass further embodiments of the invention. These cells encompass any potential cell into which the aforementioned nucleic acid might be introduced and/or transfected (for example, bacteria, COS cells, Vero cells, chick embryo fibroblasts, tumor cells, and antigen presenting cells). The choice of process for the introduction and/or transfection into cells is dependant upon the intrinsic nature of the nucleic acid (i.e. free DNA, plasmid, incorporated into a recombinant virus), as will be known to one skilled in the art (for example, as taught in *Current Protocols in Molecular Biology,* F.M. Ausubel et al. (Eds.), John Wiley and Sons, Inc., N.Y., U.S.A. (1998), Chpt. 9; *Molecular Cloning: A Laboratory Manual (2nd Ed.),* J. Sambrook, E.F. Fritsch and T. Maniatis (Eds.), Cold Spring Harbor Laboratory Press, *N.Y.,* U.S.A. (1989), Chpt's. 1,2, 3 and 16).

It is well documented that the class I and class II proteins of the major histocompatibility complex (MHC) perform a central immunological function in focusing T-lymphocytes of the immune system (i.e. CD8+ and CD4+ T lymphocytes). MHC class I proteins are expressed in nearly all nucleated cell types throughout the human body; MHC class II molecules are expressed mainly on antigen-presenting cells (APCs; namely, mononuclear phagocytes, Langerhans-dendritic cells, and B lymphocytes). These distinct classes of cell surface molecules (i.e. class I and class II) present peptides/epitopes (derived from intracellular processing of protein antigens) to T lymphocytes (CD8+ and CD4+ T lymphocytes

respectively) thus initiating both cellular and humoral immune responses. Generally, epitopes/peptides derived from alloantigens, tumor antigens or viruses will be presented in association, with MHC class I molecules; extracellular antigens/proteins will be presented in association with MHC class II molecules. However, in some contexts endogenous antigens can also be presented in association with MHC class II molecules. [These general immunological principles are well described in the art-as, for example, in *Encyclopedia of Immunology (2nd Ed.),* Peter J. Delves (Ed.-in-Chief), Academic Press, San Diego, U.S.A., pp. 174-8, 191-8, 1108-13, 1690-709(1998).] '

As such, embodiments of the invention contemplate cells into which has been introduced/transfected a nucleic acid coding for a CEA agonist polypeptide/protein wherein said cells express said polypeptide/protein.

As further conceived herein, embodiments of the invention also encompass cells into which has been introduced/transfected a nucleic acid coding for CEA agonist polypeptide/protein wherein said cells also express a MHC HLA molecule (i.e. class I and/or class II). In further embodiments, these cells are antigen-presenting cells, possibly selected from the group consisting of mononuclear phagocytes, Langerhans dendritic cells ("dendritic cell(s)"), and B lymphocytes.

Aspects of this invention contemplate mixtures of the CEA agonist polypeptide(s)/protein(s), nucleic acids coding therefor, vectors comprising said nucleic acids, or cells comprising said nucleic acids, and at least one members selected from the group consisting of cytokines, lymphokines, immunostimulatory molecules, and nucleic acids coding therefor. Additional embodiments of this invention further encompass pharmaceutical compositions comprising the CEA agonist polypeptide/protein, nucleic acids coding therefor, vectors cells or mixtures for administration to subjects in a biologically compatible form suitable for administration *in vivo*. By "biologically compatible form suitable for administration *in vivo*" is meant a form of the substance to be administered in which any toxic effects are outweighed by the therapeutic effects. Administration of a therapeutically

active amount of the pharmaceutical compositions of the present invention, or an "effective amount", is defined as an amount effective at dosages and for periods of time, necessary to achieve the desired result of eliciting an immune response in a human. A therapeutically effective amount of a substance may vary according to factors such as the disease state/health, age, sex, and weight of the recipient, and the inherent ability of the particular polypeptide, nucleic acid coding therefor, or recombinant virus to elicit a desired immune response. Dosage regima may be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or on at periodic intervals, and/or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

The compositions described herein can be prepared by *per* se known methods for the preparation of pharmaceutically acceptable compositions which can be administered to subjects, such that an effective quantity of the active substance (i.e. CEA agonist polypeptide/protein, nucleic acid coding therefor) is combined in a mixture with a pharmaceutically acceptable vehicle. Suitable vehicles are described, for example, in "Handbook of Pharmaceutical Additives" (compiled by Michael and Irene Ash, Gower Publishing Limited, Aldershot, England (1995)). On this basis, the compositions include, albeit not exclusively, solutions of the substances in association with one or more pharmaceutically acceptable vehicles or diluents, and may be contained in buffered solutions with a suitable pH and/or be iso-osmotic with physiological fluids. In this regard, reference can be made to U.S. Patent No. 5,843,456. These compositions may further comprise an adjuvant (as described below).

Methods of inducing or eliciting an immune response in an animal directed against:

a CEA protein or fragment thereof; and/or

a CEA agonist polypeptide/protein of the invention; and/or

a CEA epitope; and/or

a modified CEA epitope; and/or

cells expressing a CEA protein or fragment thereof, CEA agonist

polypeptide/protein of the invention, CEA epitope, modified CEA epitope; and/or cells binding a CEA protein or fragment thereof, CEA agonist polypeptide/protein of the invention, CEA epitope, modified CEA epitope, comprising the step of; administering to said animal a CEA agonist polypeptide/protein or fragment thereof, a nucleic acid coding therefor, a vector or cell comprising said nucleic acid; mixtures thereof or pharmaceutical compositions of the aforementioned (hereinafter collectively referred to as "immunizing agent's)", "agent's)', or "immunogen(s)") are also within the scope of this invention. As previously noted, an "immune response" is defined as any response of the immune system, for example, of either a cell-mediated (i.e. cytotoxic T-lymphocyte mediated) or humoral (i.e. antibody mediated) nature. These immuhe responses can be assessed by a number of *in vivo* or *in vitro* assays well known to one skilled in the art (for example, (but not limited to) antigen specific cytotoxicity assays, production of cytotoxins, regression of tumors expressing CEA/CEA-epitopes, inhibition of cancer cells expressing CEA/CEA epitopes).

Further embodiments of the invention encompass methods inhibiting a CEA epitope expressing carcinoma cell, in a patient comprising administering to said patient an effective amount of an immunogen of the invention. Patients with solid tumors expressing CEA (or epitopes thereof) include (but are not limited to) those suffering from colon cancer, lung cancer, pancreas cancer, endometrial cancer, breast cancer, thyroid cancer, melanoma, oral cancer, laryngeal cancer, seminoma, hepatocellular cancer, bile duct cancer, squamous cell carcinoma, and prostate cancer. As such, methods of treating patients with cancer *per* se encompassing the aforementioned methods of inducing an immune response and/or inhibiting a CEA epitope expressing carcinoma cell are contemplated aspects/embodiments of the invention.

As known to one of ordinary skill in the art, an animal may be immunized with an immunogen of the invention by any conventional route. This may include, for example, immunization via a mucosal surface (e.g., ocular, intranasal, oral, gastric, pulmonary, intestinal, rectal, vaginal, or urinary tract) or via a parenteral

route (e.g., subcutaneous, intradermal, intramuscular, intravenous, or intraperitoneal). Preferred routes depend upon the choice of the immunogen (i.e. polypeptide vs. nucleic acid, recombinant/virus, composition formulation, etc.). The administration can be achieved in a single dose or repeated at intervals. The appropriate dosage is dependant on various parameters understood by the skilled artisans, such as the immunogen itself (i.e. polypeptide vs. nucleic acid (and more specifically type thereof)), the route of administration and the condition of the animal to be vaccinated (weight, age and the like). As such, embodiments of this invention encompass methods of eliciting immune responses in animals comprising administering an effective amount of a CEA agonist polypeptide/proteins of the invention, a nucleic acid coding therefore, vector or cells or recombinant virus comprising said nucleic acid, mixtures thereof, or pharmaceutical compositions of the aforementioned.

As previously noted, nucleic acids (in particular plasmids and/or free/naked DNA and/or RNA coding for the CEA agonist polypeptide/protein of the invention) can be administered to an animal for purposes of inducing/eliciting an immune response (for example, U.S. Patent No. 5589466; McDonnell and Askari, NEJM 334:42-45 (1996), Kowalczyk and Ertl, *Cell Mot Life* Sct 55:751-770 (1999)). Typically, this nucleic acid is a form that is unable to replicate in the target animal's cell and unable to 'integrate in said animal's genome. The DNA/RNA molecule encoding the CEA agonist polypeptide/protein is also typically placed under the control of a promoter suitable for expression in the animal's cell. The promoter can function ubiquitously or tissue specifically. Examples of non-tissue specific promoters include the early Cytomegalovirus (CMV) promoter (described in U.S. Patent No. 4,168,062) and the Rous Sarcoma Virus promoter. The desmin promoter is tissue-specific and drives expression in muscle cells. More generally, useful vectors have been described (i.e., WO 94/21 797).

For administration of nucleic acids coding for a CEA agonist polypeptide/protein of the invention, said nucleic acid can encode a precursor or mature form of the polypeptide/protein. When it encodes a precursor form, the

precursor form can be homologous (for example, see Oikawa, S. et al. (1987) *Supra)* or heterologous. In the latter case, a eucaryotic leader sequence can be used, such as the leader sequence of the tissue-type plasminogen factor (tPA).

For use as an immunogen, a nucleic acid of the invention can be formulated according to various methods known to a skilled artisan. First, a nucleic acid can be used in a naked/free form, free of any delivery vehicles (such as anionic liposomes, cationic lipids, microparticles, (e.g., gold microparticles), precipitating agents (e.g., calcium phosphate) or any other transfection-facilitating agent. In this case the nucleic acid can be simply diluted in a physiologically acceptable solution (such as sterile saline or sterile buffered saline) with or without a carrier. When present, the carrier preferably is isotonic, hypotonic, or weakly hypertonic, and has a relatively low ionic strength (such as provided by a sucrose solution (e.g., a solution containing 20% sucrose)).

Alternatively, a nucleic acid can be associated with agents that assist in cellular uptake. It can be, i.e., (i) complemented with a chemical agent that modifies the cellular permeability (such as bupivacaine; see, for example, WO 94/16737), (ii) encapsulated into liposomes, or (iii) associated with cationic lipids or silica, gold, or tungsten microparticles.

Cationic lipids are well known in the art and are commonly used for gene delivery. Such lipids include Lipofectin (also known as DOTMA (N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride), DOTAP (1 ,2-bis(oleyloxy)-3-(trimethylammonio)propane). DDAB (dimethyldioctadecyl-ammonium bromide), DOGS (dioctadecylàmidologlycyl spermine) and cholesterol derivatives such as DC-Chol (3 beta-(N-(N',N'-dimethyl aminomethane)-carbarnoyl) cholesterol). A description of these cationic lipids can be found in EP 187,702, WO 90/11092, U.S. Patent No. 5,283,185, WO 91/15501, WO 95/26356, and U.S. Patent No. 5,527,928. Cationic lipids for gene delivery are preferably used in association with a neutral lipid such as DOPE (dioleyl phosphatidylethanolamine), as for example, described in WO 90/11092.

Other transfection-facilitating compounds can be added to a formulation cdntaining cationic liposomes. A number of them are described in, for example, WO 93/18759, WO 93/19768, WO 94/25608, and WO 95/2397. They include, i.e., Spermine derivatives useful for facilitating the transport of DNA through the nuclear membrane (see, for example, WO 93/18759) and membrane-permeabilizing compounds such as GALA, Gramicidine 5, and cationic bile salts (see, for example, WO 93/19768).

Gold or tungsten microparticles can also be used for gene delivery (as described in WO 91/359 and WO 93/17706). In this case, the microparticle-coated polynucleotides can be injected via intradermal or intraepidermal routes using a needleless injection device ("gene gun"), such as those described, for example, in U.S. Patent No. 4,945,b50, U.S. Patent No. 5,015,580, and WO 94/24263.

Anionic and neutral liposomes are also well-known in the art (see, for example, Liposomes: A Practical Approach, RPC New Ed, IRL Press (1990), for a detailed description of methods for making liposomes) and are useful for delivering a large range of products, including nucleic acids.

Particular embodiments of the aforementioned methods (i.e. to induce/elicit immune responses and/or to inhibit a CEA epitope expressing carcinoma cell in a patient) encompass prime-boost protocols for the administration of immunogens of the invention. More specifically, these protocols encompass (but are not limited to) a "priming" step with a particular/distinct form of immunogen (i.e. nucleic acid (for example, plasmid, bacterial/viral/free or naked) coding for an immunogen, or vector (i.e. recombinant virus, bacteria) comprising said nucleic acid) followed by at least one "boosting" step encompassing the administration of an alternate (i.e. distinct from that used to "prime") form of the immunogen (i.e. protein or fragment thereof (for example, epitope-peptide), nucleic acid coding for an immunogen (or fragment thereof), or vector comprising said nucleic acid). Examples of "prime-boost" methodologies are known to those skilled in the art (as taught, for example, in PCT

published applications WO 00/00216, WO 98/58956, WO 98/56919, WO 97/39771). One advantage of said protocols is the potential to circumvent the problem of generating neutralizing immune responses to viral vectors *per* se wherein is inserted incorporated nucleic acids encoding the immunogen or fragments thereof (see for example, R.M. Conty et al. (2000) C*lin. Cancer* Res. 6:34-41).

As is well known to those of ordinary skill in the art, the ability of an immunogen to induce/elicit an immune response can be improved if, regardless of administration formulation (i.e. recombinant virus, nucleic acid, polypeptide), said immunogen is coadministered with an adjuvant. Adjuvants are described and discussed in "Vaccine Design-the Subunit and Adjuvant Approach" (edited by Powell and Newman, 'Plenum Press, New York, U.S.A., pp. 61-79 and 141-228 (1995)). Adjuvants typically enhance the immunogenicity of an Immunogen but are not necessarily immunogenic in and of themselves. Adjuvants may act by retaining the immunogen locally near the site of administration to produce a depot effect facilitating a slow, sustained release of immunizing agent to cells of the immune system. Adjuvants can also attract cells of the, immune system to an immunogen depot and stimulate such cells to elicit immune responses. As such, embodiments of this invention encompass compositions further comprising adjuvants.

Desirable characteristics of ideal adjuvants include:

1) lack of toxicity:
2) ability to stimulate a long-lasting immune response;
3) simplicity of manufacture and stability in long-term storage;
4) ability to elicit both cellular and humoral responses to antigens administered by various routes, if required:
5) synergy with other adjuvants;
6) capability of selectively interacting with populations of antigen presenting cells (APC);
7) ability to specifically elicit appropriate TRI or TH2 cell-specifib immune responses; and
8) ability to selectively increase appropriate antibody isotype levels (for

example, IgA) against antigens/immunogens.

However, many adjuvants are toxic and can cause undesirable side effects, thus making them unsuitable for use in humans and many animals. For example, some adjuvants may induce granulomas, acute and chronic inflammations (i.e. Freund's complete adjuvant (FCA)), cytolysis (i.e. saponins and pluronic polymers) and pyrogenicity, arthritis and anterior uveitis (i.e. muramyl dipeptide (MDP) and lipopolysaccharide (LPS)). Indeed, only aluminum hydroxide and aluminum phosphate (collectively commonly referred to as alum) are routinely used as adjuvants in human and veterinary vaccines. The efficacy of alum in increasing antibody responses to diphtheria and tetanus toxoids is well established. Notwithstanding, it does have limitations. For example, alum is ineffective for influenza vaccination and inconsistently elicits a cell mediated immune response with other immunogens. The antibodies elicited by alum-adjuvanted antigens are mainly of the IgG1 isotype in the mouse, which may not be optimal for protection in vaccination contexts.

Adjuvants may be characterized as "intrinsic" or "extrinsic". Intrinsic adjuvants (such as lipopolysaccharides) are integral and normal components of agents which in themselves are used as vaccines (i.e. killed or attenuated bacteria), Extrinsic adjuvants are typically nonintegral immunomodulators generally linked to antigens in a noncovalent manner, and are formulated to enhance the host immune response.

In embodiments of the invention, adjuvants can be at least one member chosen from the group consisting of cytokines, lymphokines, and co-stimulatory molecules. Examples include (but are not limited to) interleukin 2, interleukin 12, interleukin 6, interferon gamma, tumor necrosis factor alpha, GM-CSF, 87.1, 87.2, ICAM-1, LFA-3, and CD72. Particular embodiments specifically encompass the use of GM-CSF as an adjuvant (as taught, for example, in U.S. Patent Nos. 5679356, 5904920, 5637483, 5759535, 5254534, European Patent Application EP 211684, and published PCT document WO 97/28816 all of which are herein incorporated by reference).

A variety of potent extrinsic adjuvants have been described. These include (but are not limited to) saponins complexed to membrane protein antigens (immune stimulating complexes), pluronic polymers with mineral oil, killed mycobacteria and mineral oil, Freund's complete adjuvant, bacterial products such as muramyl dipeptide (MDP) and lipopolysaccharide (LPS), as well as lipid A, and liposomes.

The use of saponins *per* se as adjuvants is also well known (Lacaille-Dubois, M. and Wagner, H (1996) *Phytomedicine* 2:363). For example, Quil A (derived from the bark of the South American tree, Quillaja Saponaria Molina) and fractions thereof has been extensively described (i.e. U.S. Patent No. 5057540; Kensil, C.R. (1996) *Crit* Rev *Ther Drug Carrier Syst* 12:1; and European Patent EP 362279). The haemolytic saponins Q521 and QSI7 (HPLC purified fractions of Quil A) have been described as potent systemic adjuvants (U.S. Patent No. 5057540: European Patent EP 362279). Also described in these references is the use of QS7 (a non-haemolytic fraction of Quil-A) which acts as a potent adjuvant for systemic vaccines. Use of QS2I is further described in Kensil et al. ((1991) *J. Immunol* 146:431). Combinations of O521 and polysorbate or cyclodextrin are also known (WO 9910008). Particulate adjuvant systems comprising fractions of Quil A (such as QS2I and QS7) are described in WO 9633739 and WO 9611711.

Another preferred adjuvant/immunostimulant is an immunostimulatory oligonucleotide containing unmethylated CpG dinucleotides ("CpG"). CpG is an abbreviation f.or cytosine-guanosine dinucleotide motifs present in DNA. CpG is known in the art as being an adjuvant when administered by both systemic and mucosal routes (WO 9602555; European Patent EP 468520; Davies et al. (1998). *J. Immunol.* 160:87; McCluskie and Davis (1998) *J. Immunol* 161:4463). In a number of studies, synthetic oligonucleotides derived from BCG gene sequences have also been shown to be capable of inducing immunostimulatory effects (both *in vitro* and *in vivo*; Krieg, (1995) *Nature* 374:546). Detailed analyses of immunostimulatory oligonucleotide sequences has demonstrated that the CG motif must be in a certain sequence context, and that such sequences are common in bacterial DNA but are

rare in vertebrate DNA. (For example, the immunostimulatory sequence is often: purine, purine, C, G, pyrimidine, pyrimidine, wherein the CG motif is not methylated; however other unmethylated CpG sequences are known to be immunostimulatory and as such may also be used in the present invention.) As will be evident to one of normal skill in the art, said CG motifs/sequences can be. incorporated into nucleic acids of the invention *per se,* or reside on distinct nucleic acids.

A variety of other adjuvants are taught in the art, and as such are encompassed by embodiments of this invention. U.S. Patent No. 4,855,283 granted to Lockhoff et al. (incorporated herein by reference) teaches glycolipid analogues and their use as adjuvants. These include N-glycosylamides, N-glycosylureas and N-glycosylcarbamates, each of which is substituted in the sugar residue by an amino acid, as immuno-modulators or adjuvants. Furthermore, Lockhoff et al. ((1991) *Chem. mt. Ed. EngI.* 30:1611) have reported that N-glycolipid analogs displaying structural similarities to the naturally-occurring glycolipids (such as glycophospholipids and glycoglycerolipids) are also capable of eliciting strong immune responses in both herpes simplex virus vaccine and pseudorabies virus vaccine.

U.S. Patent No. 4,258,029 granted to Moloney (incorporated herein by reference) teaches that octadecyl tyrosine hydrochloride (OTH) functions as an adjuvant when complexed with tetanus toxoid and formalin inadtivated type I, II and III poliomyelitis virus vaccine. Nixon-George et al. ((1990) *J. Immunol.* 14:4798) have also reported that octadecyl esters of aromatib amino acids complexed with a recombinant hepatitis B surface antigen enhanced the host immune responses against hepatitis B virus.

Adjuvant compounds may also be chosen from the polymers of acrylic or methacrylic acid and the copolymers of maleic anhydride and alkenyl derivative. Adjuvant compounds are the polymers of acrylic or methacrylic acid which are cross-linked, especially with polyalkenyl ethers of sugars or polyalcohols. These

compounds are known by the term carbomer (Pharmeuropa Vol. 8, No. 2, June 1996). Preferably, a solution of adjuvant according to the invention, especially of carbomer, is prepared in distilled water, preferably in the presence of sodium chloride, the solution obtained being at acidic pH. This stock solution is diluted by adding it to the desired quantity (for obtaining the desired final concentration), or a substantial part thereof, of water charged with NaCI, preferably physiological saline (NaCl 9g/l) all at once in several portions with concomitant or subsequent neutralization (pH 7.3 to 7.4), preferably with NaOH. This solution at physiological pH will be used as it is for mixing with the immunizing agent; said mixture being amenable to storage in the freeze-dried, liquid or frozen form.

Persons skilled in the art can also refer to U.S. Patent No. 2,909,462 (incorporated herein by reference) which describes adjuvants encompassing acrylic polymers cross-linked with a polyhydroxylated compound having at least 3 hydroxyl groups (preferably not more than 8), the hydrogen atoms of the at least three hydroxyls being replaced by unsaturated aliphatic radicals having at least 2 carbon atoms. The preferred radicals are those containing from 2 to 4 carbon atoms (e.g. vinyls, allyls and other ethylenically unsaturated groups). The unsaturated radicals may themselves contain other substituents, such as methyl. The products sold under the name Carbopol (BF Goodrich, Ohio, USA) are particularly appropriate. They are cross-linked with allyl sucrose or with allyl pentaerythritol. Among them, there may be mentioned Carbopol (for example, 974P, 934P and 971 P). Among the copolymers of maleic anhydride and alkenyl derivative, the copolymers EMA (Monsanto; which are copolymers of maleic anhydride and ethylene, linear or cross-linked, (for example cross-linked with divinyl ether)) are preferred. Reference may be made to J. Fields et al. ((1960) *Nature* 186:778) for a further description of these chemicals (incorporated (herein by reference).

In further aspects of this invention, adjuvants useful for parenteral administration of immunizing agent include aluminum compounds (such as aluminum hydroxide, aluminum phosphate, and aluminum hydroxy phosphate; but might also be a salt of calcium, iron or zinc, or may be an insoluble suspension of

acylated tyrosine, or acylated sugars, cationically or anionically derivatised polysaccharides, or polyphosphazenes). The antigen can be precipitated with, or adsorbed onto, the aluminum compound according to standard protocols well known to those skilled in the art.

Other adjuvants encompassed by embodiments of this invention include lipid A (in particular 3-de-0-acylated monophosphoryl lipid A (3D-MPL). 3D-MPL is a well known adjuvant manufactured by Ribi Immunochem, Montana. Chemically it is often supplied as a mixture of 3-de-0-acylated monophosphoryl lipid A with 4, 5, or 6 acylated chains. It can be prepared by the methods taught in GB 21222048. A preferred form of 3D-MPL is in the form of a particulate formulation having a particle size less than 0.2pm in diameter (European Patent EP 689454).

Adjuvants for mucosal immunization may include bacterial toxins (e.g., the cholera toxin (CT), the *E. coli* heat-labile toxin (LT), the *Clostrilium difficile* toxin A and the pertussis toxin (PT), or combinations, subunits, toxoids, or mutants thereof). For example, a purified preparétion of native cholera toxin subunit B (CTB) can be of use. Fragments, homologs, derivatives, and fusion to any of these toxins are also suitable, provided that they retain adjuvant activity. A mutant having reduced toxicity may be used. Mutants have been described (e.g., in WO 95/17211 (Arg-7-Lys CT mutant), WO 96/6627 (Arg 192-Gly LT mutant), and WO 95134323 (Arg-9-Lys and Glu-129-Gly PT mutant)). Additional LT mutants include, for example Ser-63-Lys, Ala-69-Gly, Glu-1 10-Asp, and Glu-1 12-Asp mutants. Other adjuvants (such as a bacterial monophosphoryl lipid A (MPLA)) of various sources (e.g., *E. coli, Salmone/Ia minnesota, Salmonella typhimurium,* or *Shigella flexneri)* can also be used in the mucosal administration of immunizing agents.

Adjuvants useful for both mucosal and parenteral immunization include polyphosphazene (for example, WO 95/2415), DC-chol (3 b-(N-(N',N'-dimethyl aminomethane)-carbamoyl) cholesterol (for example, U.S. Patent No. 5,283,185 and WO 96/14831) and QS-21 (for example, WO 88/9336).

Adjuvants/immunostimulants as described herein may be formulated together with carriers, such as for example liposomes, oil in water emulsions, and/or metallic salts including aluminum salts (such as aluminum hydroxide). For example, 3D-MPL may be formulated with aluminum hydroxide (as discussed in EP 689454) or oil in water emulsions (as discussed in WO 9517210); QS2I may be advantageously formulated with cholesterol containing liposomes (as discussed in WO 9633739), in oil water emulsions (as discussed in WO 9517210) or alum (as discussed in WO 9815287). When formulated into vaccines, immunostimulatory oligonucleotides (i.e. CpGs) are generally administered in free solution together with free antigen (as discussed in WO 9602555; McCluskie and Davis (1998) *Supra),* covalently conjugated to an antigen (as discussed in WO 9816247), or formulated with a carrier such as aluminum hydroxide or alum (as discussed in Davies et al. *Supra;* Brazolot-Millan et al (1998) *Proc. Natl. Acad. Sci.* 95:15553).

Combinations of adjuvants/immunostimulants are also within the scope of this invention. For example, a combination of a monophosphoryl lipid A and a saponin derivative (as described in WO 9400153, WO 9517210, WO 9633739, WO 9856414, WO 9912565, WO 9911214) can be used, or more particularly the combination of QS21 and 3D-MPL (as described in WO 9400153). A combination of an immunostimulatory oligonUcleotide and a saponin (such as QS2I), or a combination of monophosphoryl lipid A (preferably 3D-MPL) in combination with an aluminum salt also form a potent adjuvant for use in the present invention.

The following non-limiting examples are illustrative of the present invention:

EXAMPLES

Example 1: Generation of a Modified CEA Gene

T-cell epitopes within the native CEA protein include CAP-1; said epitope comprising the amino acid sequence YLSGANLNL (Tsang et al. (1995) *J. Natl. Cancer Inst* 87:982-990). As previously noted, the immunogenicity of this epitope (in the form of a peptide) was increased by changing position six of the CAP-1

epitope from N (asparagine) to D (aspartic acid) (Zaremba et al. (1997) *Cancer Res.* 57:4570-4577). Using standard techniques of *in vitro* mutagenesis (Ausubel et al. (1997) *Curr. Protocols in Mol. Blot),* this modification was introduced into the full-length CEA gene via mutation of the codon AAC (which encodes asparagine) to GAC (which encodes aspartic acid). The resulting modified CEA gene now comprises the modified epitope YLSGADLNL (the change from N to D is shown in bold). This modified CEA gene, encoding for a protein with aspartic acid (D) in place of asparagine (N) in the sixth amino acid position of the CAP-1 epitope, is designated CEA (6D) (for example, see SEQ ID NO: 1 (Figure 1)).

Example 2: Generation of Recombinant Fowlpox, Vaccinia and MVA Constructs

The generation of recombinant poxviruses is accomplished via homologous recombination between poxvirus genomic DNA and a plasmid vector that carries the heterologous sequences to be inserted. Plasmid vectors for the insertion of foreign sequences into poxviruses are constructed by standard methods of recombinant DNA technology (for example, Ausubel et al. (1997) *Curr. Protocols. Mol. Biol).* The plasmid vectors contain one or more chimeric genes, each comprising a poxvirus promoter linked to a protein coding sequence, flanked by viral sequences from a non-essential region of the poxvirus genome. The plasmid is transfected into cells infected with the parental poxvirus, and recombination between poxvirus sequences on the plasmid and the corresponding DNA in the viral genome results in the insertion into the viral genome of the chimeric genes on the plasmid. Recombinant viruses are selected and purified using any of a variety of selection or screening systems (Mazzara et al. (1993) *Meth. Enzymol.* 217:557-581; Jenkins et al. (1991) *AIDS Res. Hum. Retroviruses* 7:991-998; Sutter et al. (1994) *Vaccine* 12:1032-1040). Insertion of the foreign genes into the vaccinia genome is typically confirmed by polymerase chain reaction (PCR) analysis. Expression of the foreign genes is demonstrated by Western analysis and/or via immunoprecipitation of expressed products.

Generation of Recombinant Poxviruses

For the generation of recombinant fowlpox virus rF-CEA(6D), a plasmid vector designated pT5071 was constructed to direct insertion of the foreign sequences into the BamHl J region of the fowlpox genome. The CEA(6D) gene is under the control of the vaccinia 40K promoter (Gritz et al. (1990) ***J. Virol.*** 64:5948-5957). In addition, the *E. coil lacZ* gene, under the control of the fowlpox virus C1 promoter (Jenkins et al. (1991) *AIDS* Res. *Hum. Retroviruses* 7:991-998), is included as a screen for recombinant progeny. These foreign sequences are flanked by DNA sequences from the BamHI J region of the fowlpox genome. A plaque-purified isolate from the POXVAC-TC strain of fowlpox was used as the parental virus for this recombinant vaccine. The geheration of recombinant fowlpox virus was accomplished via homologous recombinant between fowlpox sequences in the fowlpox genome and the corresponding sequences in pT5071 in fowlpox-infected primary chick embryo dermal cells transfected with pT5O71. Recombinant virus was identified using a chromogenic assay preformed on viral plaques *in situ.* This assay detects expression of the lacZ gene product in the presence of halogenated indolyl-beta-D-galactoside (Bluo-gal) (Chakrabarti et al. (1985) *Mol. Cell Bio* 3403-3409). Viral plaques expressing *IacZ* appear blue against a clear background. Positive plaques, designated vT233, were picked from the cell monolayer and their progeny replated. Seven rounds of plaque isolation and replating in the presence of Bluo-Gal resulted in the purification of the desired recombinant. A schematic representation of the genomic structure of vT233 is depicted in Figure 2(A).

For the generation of recombinant vaccinia rV-CEA(6D), a plasmid vector designated pT2 146 was constructed to direct insertion of the foreign sequences into the thymidine kinase gene (located in the Hind Ill J region of the vaccinia genome). The CEA(6D) gene is under the transcriptional control of the vaccinia 40K promoter and the *E. coIl. IacZ* gene is under the control of the fowlpox virus Ci promoter. These foreign sequences are flanked by DNA sequences from the Hind Ill J region of the vaccinia genome. A plaque-purified isolate from the Wyeth (New York City Board of Health) strain of vaccinia was used as the parental virus for this recombinant vaccine. The generation of recombinant vaccinia virus was

accomplished via homologous recombination between vaccinia sequences in the Wyeth vaccinia genome and the corresponding sequences in pT2146 in vaccinia-infected cells transfected with pT2146. Recombinant virus was identified using the chromogenic assay for the *lacZ* gene product (previously described). Positive plaques, designated vT237, were picked from the cell monolayer and their progeny replated. Five rounds of plaque isolation and replating in the presence of Bluo-Gal resulted in the purification of the desired recombinant. A schematic representation of the genomic structure of vT237 is depicted in Figure 2(6).

For the generation of a recombinant MVA that expressed CEA(6D), a plasmid vector was constructed to direct insertion of the foreign sequences into the MVA genome. The CEA(6D) gene and the *E. coil* lacZ gene were each under the control of a poxviral promoter. These foreign sequences were flanked by DNA sequences from the MVA genome into which the foreign sequences were to be inserted (for example, deletion Ill (Sutter et at (1994) Vaccine 12:1032-1040)). The generation of recombinant MVA was accomplished via homologous recombination between MVA sequences in the MVA gehome and the corresponding sequences in the plasmid vector in MVA-infected cells transfected withthe plasmid vector. Recombinant plaques were picked from the cell monolayer under selective conditions and their progeny further propagated. Additional rounds of plaque isolation and replating resulted in the purification of the desired recombinant virus. A schematic representation of the genomic structure of the MVA recombinant construct is depicted in Figure 2(C).

Example 3: Generation of anAI_VAC(2)-modified CEA-B7.1 (Human) Recombinant Construct frCP1586)

To generate ALVAC(2)-CEAmod/hB7.1, a coding sequence containing the H6 (Perkins et al. (1989) *J. Virol.* 63:3829-3936) promoted modified CEA and the human 67.1 gene (under the control of synthetic Early/Late promoter) was subcloned into a generic ALVAC donor plasmid specific for the C5 insertion site (see below). The donor plasmici was then used to insert the expression cassette into

the CS insertion locus in the ALVAC(2) genome by *in vitro* recombination (Piccini et al. (1987) *Meth. Enzymol.* 153:545).

The locus designated CS was used for the insertion of the modified CEA and human B7.1 coding sequences into the ALVAC(2) vector. By virtue of the C5 locus existing within the extensive inverted terminal repetitions (ITRs) of the virus genome, insertion into this locus results in the occurrence of two copies of the inserted sequence. A schematic of the redombinant construct is depicted in Figure 3. [Presently, no function has been ascribed to the CS encoded polypeptide nor does the deduced amino acid open reading frame encoded in this region share significant homology to any entry in the existing protein databases.]

Particulars of the Generation of the vCPIS86 ALVAC recombinant

(I) Modified CEA:
sequence (up to the Bg/II site) by PCR amplification from plasmid pT2147 (comprising a full length sequence of the modified CEA(6D)) using the following PCR primers:

HM102: 5 '-TTG-TCC-GAG-CTC-TCG-CGA-TAT-CCG-TTA-AGT-TTG-TAT CGT-AAT-GGA-GTC-TCC-CTC-GGC-CCC-3' (Sequence ID No. 3)

HM103: 5'-CCG-GAA-TTC-TCA-CAA-GAT-CTG-ACT-TTA-TGA-C-3' (Sequence ID NO. 4)

The PCR product was digested with *Sac*I and *Eco*Rl to generate compatible ends for cloning into *Sac*I, *Eco*Rl and Shrimp Alkaline Phosphatase digested pBSK+ vector (DH5α cells). The resulting plasmid (designated pF102.103) was sequenced to confirm the fidelity of the insert. The remainder of the CEA(6D) sequence was isolated by digestion of plasmid pT2147 with *Bg/*II and Sa/I. The resulting 1.7Kbp fragment contained the 3'end of CEA(6D). This fragment was ligated to *Bg/*II, *Sal*I

and Shrimp Alkaline Phosphatase digested pF102.103 plasmid and transformed into DH5α cells. The resulting plasmid was designated p3'H6MCEA.

An ALVAC donor plasmid was constructed by transferring the 3'H6-modified CEA sequence fragment from p3'H6MCEA to ALVAC insertion plasmid NVQH6MC5. NVQH6MC5 was initially made via digestion of CS donor plasmid NVQH6C5LSP-18 within its polylinker region with *BamHl.* It was subsequently treated with alkaline phosphatase and ligated to kinased and annealed oligonucleotides SPCSPL1 (5'-GAT-CGT-CGA-CGA-GCT-CGA-ATT-CG-3') (Sequence ID No. 5) and SPC5PL2 (S'-GAT-CCG-AATTCG-ACC-TCG-TCG-AC-3') (Sequence ID No. 6) - thus generating plasmid NVQH6MC5.

Plasmid p3'H6MCEA was digested with *Nru*I and *SalI;* the fragment containing the 3'H6 modified CEA sequence purified and subsequently ligated to *Nru*I, *Xho*I and Shrimp Alkaline Phosphatase digested NVQH6MC5 vector. The resulting donor plasmid, containing the modified CEA coding sequences under the control of a full length H6 promoter, was designated pAH6MCEA.

(ii) B7.1

The following primers were used to fuse the 42K promoter and PCR out the entire B7.1 gene from plasmid pT2147:

H Ml 04: 5'-TTG-TCC-GAG-CTC-GAA-TTC-TTT-ATT-G GG-AAG-AAT-ATG-ATA-ATA-TTT-TGG-GAT-TTC-AAA-ATT-GAA-AAT-ATA-TAA-TTACAA-TAT-AAA-ATG-GGC-CAC-ACA-CGG-AGG-CAG-3' (Sequence ID No. 7).
HMI05: 5'-ACG-GCA-GTC-GAC-TTA-TAC-AGG-GCG-TAC-ACT-3' (Sequence ID No. 8).

Primer HM1O4 contained the entire sequence of the 42K promoter. The resulting PCR product (designated F104.105) was digested with *Eco*RI and *Sa/I,* and

subsequently ligated into *Eco*RI, *Sa*II and Shrimp Alkaline Phosphatase digested pBSK+ vector(SURE cells), The resulting plasmid was designated pFl04.105 and was sequenced to confirm the fidelity of the insert.

(iii) Donor Plasmid pA2147

The fragment containing the 42K-67.1 coding sequences was excised from pFl04.105 by digestion with *Eco*RI and *Sa/I,* and subsequently purified. This fragment was ligated to *Eco*RI, *Sa/I,* Shrimp Alkaline Phosphatase digested pAH6MCEA plasmid and transformed into SURE cells. The resulting donor plasmid was designated pA2147. (See Figure 4 for the sequence of the H6-promoted human modified CEA/42K promoted B7.1 cassette of pA2147 for insertion into ALVAC.)

(iv) Generation of VCP1586

Recombination between donor plasmid pA2147 and ALVAC(2) rescuing virus generated recombinant virus vCP1586. This recombinant viral construct comprised the vaccinia H6 promoted modified human CEA gene sequence and the 42K promoted human 67.1 gene sequence in the C5 locus (for ALVAC insertion and inserted DNA sequence particulars, see Figures 3 and 4). Recombination was performed utilizing procedures described in the art and known to skilled artisans (Piccini et al. (1987), *supra;* other procedures can also be utilized as described above and/or as described, for example, in U.S. Patent Nos. 4769330, 4722848, 4603112, 5174993, 5110587, all of which are incorporated herein by reference).

Verification of insertion

(I) Restriction Enzyme Analysis

Viral genomic DNA was isolated from cells infected with vCP1586 pursuant to methods well known to those skilled in the art (for example, as taught in *Current*

Protocols in Molecular Biology, F.M. Ausubel et al. (Eds.), John Wiley and Sons, Inc., N.Y., U.S.A. (1998); *Molecular Cloning: A Laboratory Manual (2nd Ed.),* J. Sambrook, E.F. Fritsch and T. Maniatis (Eds.), Cold Spring Harbor Laboratory Press, N.Y., U.S.A. (1989)). The viral genomic DNA was digested with restriction endonucleases *(HindIII,* Pst I, BamHl or *Xhol).* The resultant DNA fragments were fractionated by electrophoresis through an agarose gel and visualized by ethidium bromide staining. The insertion of the modified CEA and B7.1 expression cassette at the CS locus was confirmed (for example, see Figure 3 for a schematic representation of the *Xhol* restriction map of vCPI 586).

(ii) Immunoprecipitation

Immunoprecipitation analyses were performed using radiolabeled lysates derived from uninfected HeLa cells or cells infected with either ALVAC(2) parental virus (vCP1486), ALVAC-CEA/B7 (vCP3O7), ALVAC-hB7.1 (vCP11334), ALVAC-CEAmod/hB7.1 (vCP1585) or ALVAC(2)-CEAmod/hB7.1 (vCP1586) as previously described (Taylor et al. (1990) *J. Virol.* 64:1441-1450). Briefly, HeLa cell cultures were infected at a multiplicity of infection (m.o.i.) of 10 pfu/cell in methionine and cystine-free media supplemented with [^{35}S]-methionine/cysteine (30μCi/mi). Cells were lysed at 18 hours post-infection. Immunoprecipitation was performed using a B7.1 specific monoclonal antibody (HB71; ATCC#80991). immunoprecipitates were fractionated on a 10% SDS-Polyacrylamide gel. The gel was fixed and treated for fluorography with 1 M Na-salicylate for 1/2 hr. The dried gel was exposed to Kodak XAR-2 film to visualize the protein species. Results with anti-hB7.1 demonstrate expression of human B7 in HeLa cells infected with ALVAC(2)-CEAmod/hB7.1 (vCP1586) and the other ALVAC-based recombinants expressing hB7.1, but not in parentally infected cells (Figure 5).

(iii) Western Blot Analysis

HeLa cells were infected for 18 hours at a multiplicity of 10 pfu/cell with either ALVAC(2) parental virus (vCP1486), ALVAC-CEA/B7 (vCP3O7), ALVAC-

CEA (vCP248), ALVAC-CEAmod/hB7.1 (vCP 1585) or ALVAC(2)-CEAmod/hB7. 1 (vCPI 586). Cell lysates were separated by SDS-PAGE and transferred to nitrocellulose utilizing standard techniques. The blot was incubated with anti-CEA monoclonal antibody Col-1 (1/1 000 dilution), followed by HRP conjugated rabbit anti-mouse utilizing HRP peroxide substrate with DAB/Metal (Pierce). The results obtained demonstrate the expression of full length CEA in HeLa cells infected with ALVAC(2)-CEAmod/hB7.1 (vCP1586) and other ALVAC-based recombinants expressing CEA (Figure 6).

Having illustrated and described the principles of the invention in particular and/or preferred embodiments, it should be appreciated by those skilled in the art that the invention can be modified in arrangement and detail without departure from such principles. We claim all modifications coming within the scope of the following claims. All publications, patents and patent applications referred to herein, are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

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We claim:

1. A CEA agonist polypeptide comprising a modified epitope of CEA, wherein said modified epitope contains the sequence YLSGADLNL.

2. The CEA agonist polypeptide of claim 1 having the sequence of SEQ ID NO: 1 (Figure 1).

3. A nucleic acid comprising a nucleic acid sequence which encodes the CEA agonist polypeptide of any one of claims 1 or 2.

4. The nucleic acid of claim 3 comprising the sequence of SEQ ID NO: 2 (Figure 1).

5. The nucleic acid of claim 3 having the sequence of SEQ ID NO: 2 (Figure I).

6. The nucleic acid of any one of claims 3 through 5 wherein the nucleic acid is selected from viral nucleic acid, bacterial DNA, plasmid DNA, naked/free DNA, and RNA.

7. The nucleic acid of claim 6 wherein the viral nucleic acid is selected from the group consisting of adenoviral, alphaviral, and poxviral nucleic acid.

8. The poxviral nucleic acid of claim 7, wherein said nucleic acid is selected from the group consisting of avipox, orthopox, and suipox nucleic acid.

9. The poxviral nucleic acid of claim 7, wherein said nucleic acid is selected from the group consisting of vaccinia, fowlpox, canary pox and swinepox.

10. The poxviral nucleic acid of claim 7, wherein said nucleic acid is selected from the group consisting of TROVAC, NYVAC, ALVAC, MVA, Wyeth, and Poxvac-TC.

11. The nucleic acid of any one of claims 3 through 10 further comprising a nucleic acid sequence encoding at least one member selected from the group comprising cytokines, lymphokines, and co-stimulatory molecules.

12. A vector comprising the nucleic acid of any one of claims 3 through 11.

13. The vector of claim 12, wherein said vector is selected from the group consisting of recombinant viruses and bacteria.

14. The recombinant virus vector of claim 13, selected from the group consisting of adenovirus, alphavirus and poxvirus.

15. The poxvirus of claim 14, selected from the group consisting of avipox, orthopox, and suipox.

16. The poxvirus of claim 14, selected from the group consisting of vaccinia, fowlpox, canary pox, and swinepox.

17. The poxvirus of claim 14, selected from the group consisting of TROVAC, NYVAC, ALVAC, MVA, Wyeth, and Poxvac-TC.

18. The vector of any one of claims 12 through 17 further comprising a nucleic acid sequence encoding at least one member selected from he group consisting of cytokines, lymphokines, and co-stimulatory molecules.

19. A cell comprising a nucleic acid according to any one of claims 3 through 11 wherein the cell expresses a CEA agonist polypeptide.

20. The cell of claim 19 wherein the cell additionally expresses a MHC HLA class I molecule.

21. The cell of claim 19 wherein the cell is an antigen-presenting cell.

22. The cell of claim 21 wherein the cell is a dendritic cell.

23. A mixture comprising a nucleic acid according to any one of claims 3 through 11 and at least one member selected from the group consisting of cytokines, lymphokines, immunostimulatory molecules, and nucleic acids coding therefor.

24. A mixture comprising a vector according to any one of claims 12 through 18 and at least one member selected from the group consisting of cytokines, lymphokines, immunostimulatory molecules, and nucleic acids coding therefor.

25. A mixture comprising a CEA agonist polypeptide according to any one of claims 1 or 2 and at least one member selected from the group consisting of cytokines, lymphokines, immunostimulatory molecules, and nucleic acids coding therefor.

26. A mixture comprising a cell according to any one of claims 19 through 22 and at least one member selected from the group consisting of cytokines, lymphokines, immunostimulatory molecules, and nucleic acids coding therefor.

27. A pharmaceutical composition comprising a CEA agonist polypeptide according to any one of claims 1 or 2, and a pharmaceutically acceptable diluent or carrier.

28. A pharmaceutical composition comprising a nucleic acid according to any one

of claims 3 through 11, and a pharmaceutically acceptable diluent or carrier.

29. A pharmaceutical composition comprising a vector according to any one of claims
12 through 18, and a pharmaceutically acceptable diluent or carrier.

30. A pharmaceutical composition comprising a cell according to any one of claims 19 through 22, and a pharmaceutically acceptable diluent or carrier.

31. A pharmaceutical composition comprising a mixture according to any one of claims
23 through 26, and a pharmaceutically acceptable diluent or carrier.

32. The pharmaceutical composition of any one of claims 27 through 31 further comprising an adjuvant.

33. A method of inducing an immune response in an animal directed against a member selected from the group consisting of:

a CEA protein or fragment thereof,
a CEA agonist polypeptide according to any one of claims 1 or 2,
a CEA epitope,
a modified CEA epitope,
cells expressing a CEA protein or fragment thereof, CEA agonist polypeptide according to any one of claims 1 or 2, CEA epitope, modified CEA epitope, and
cells binding a CEA protein or fragment thereof, CEA agonist polypeptide according to any one of claims 1 or 2, CEA epitope, modified CEA epitope

comprising administering to said animal a CEA agonist polypeptide or fragment thereof according to any one of claims 1 or 2 in an amount sufficient to induce an immune response.

34. A method of inducing an immune response in an animal directed against a member selected from the group consisting of:

a CEA protein or fragment thereof,

a CEA agonist polypeptide according to any one of claims 1 or 2,

a CEA epitope,

cells expressing a CEA protein or fragment thereof, CEA agonist polypeptide according to any one of claims 1 or 2, CEA epitope, modified CEA epitope, and

cells binding a CEA protein or fragment thereof, CEA agonist polypeptide according to any one of claims 1 or 2, CEA epitope, modified CEA epitope

comprising administering to said animal a nucleic acid according to any one of claims 3 through 11 in an amount sufficient to induce an immune response.

35. A method of inducing an immune response in an animal directed against a member selected from the group consisting of:

a CEA protein or fragment thereof,

a CEA agonist polypeptide according to any one of claims 1 or 2,

a CEA epitope,

a modified CEA epitope,

cells expressing a CEA protein or fragment thereof, CEA agonist polypeptide according to any one of claims 1 or 2, CEA epitope, modified CEA epitope, and

cells binding a CEA protein or fragment thereof, CEA agonist polypeptide according to any one of claims 1 or 2, CEA epitope, modified CEA epitope

comprising administering to said animal a vector according to any one of claims 12 through 18 in an amount sufficient to induce an immune response.

36. A method of inducing an immune response in an animal directed against a member selected from the group consisting of:

a CEA protein or fragment thereof,

a CEA agonist polypeptide according to any one of claims 1 or 2,
a CEA epitope,
cells expressing a CEA protein or fragment thereof, CEA agonist polypeptide according to any one of claims 1 or 2, CEA epitope, modified CEA epitope, and
cells binding a CEA protein or fragment thereof, CEA agonist polypeptide according to any one of claims 1 or 2, CEA epitope, modified CEA epitope

comprising administering to said animal a cell according to any one of claims 19 through 22 in an amount sufficient to induce an immune response.

37. A method of inducing an immune response in an animal directed against a member selected from the group consisting of:
a CEA protein or fragment thereof,
a CEA agonist polypeptide according to any one of claims 1 or 2,
a CEA epitope,
a modified CEA epitope,
cells expressing a CEA protein or fragment thereof, CEA agonist polypeptide according to any one of claims 1 or 2, CEA epitope, modified CEA epitope, and
cells binding a CEA protein or fragment thereof, CEA agonist polypeptide
according to any one of claims 1 or 2, CEA epitope, modified CEA epitope

comprising administering to said animal a mixture according to any one of claims 23 through 26 in an amount sufficient to induce an immune response.

38. A method of inducing an immune response in an animal directed against a member selected from the group consisting of:
a CEA protein or fragment thereof,
a CEA agonist polypeptide according to any one of claims 1 or 2,
a CEA epitope,

cells expressing a CEA protein or fragment thereof, CEA agonist polypeptide according to any one of claims 1 or 2, CEA epitope, modified CEA epitope, and

cells binding a CEA protein or fragment thereof, CEA agonist polypeptide according to any one of claims 1 or 2, CEA epitope, modified CEA epitope

comprising administering to said animal a composition according to any one of claims 27 through 32 in an amount sufficient to induce an immune response.

39. A method of inhibiting a CEA epitope expressing carcinoma cell in a patient comprising administering to said patient an effective amount of the CEA agonist polypeptide according to any one of claims 1 or 2.

40. A method of inhibiting a CEA epitope expressing carcinoma cell in a patient comprising administering to said patient an effective amount of the nucleic acid according to any one of claims 3 through 11.

41. A method of inhibiting a CEA epitope expressing carcinoma cell in a patient comprising administering to said patient an, effective amount of the vector according to any one of claims 12 through 18.

42. A method of inhibiting a CEA epitope expressing carcinoma cell in a patient comprising administering to said patient an effective amount of the cell according to any one of claims 19 through 22.

43. A method of inhibiting a CEA epitope expressing carcinoma cell in a patient comprising administering to said patient an effective amount of the mixture according to any one of claims 23 through 26.

44. A method of inhibiting a CEA epitope expressing carcinoma cell in a patient comprising administering to said patient an effective amount of the

composition according to any one of claims 27 through 32.

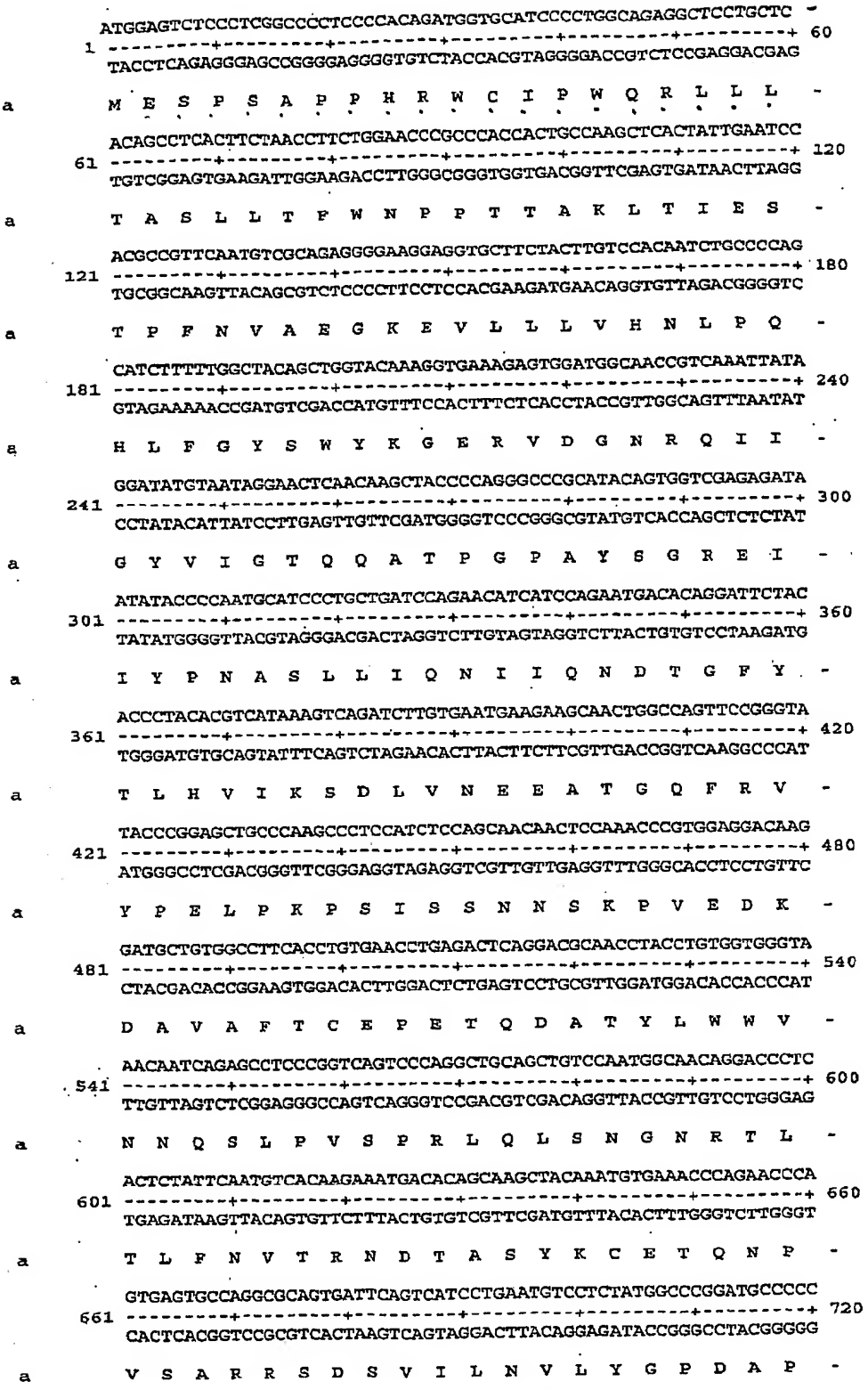
45. The methods of any one of claims 39 through 44 wherein the carcinoma cell is a gastrointestinal, breast, pancreatic, bladder, ovarian, lung or prostate carcinoma cell.

46. A treatment for cancer comprising any one of the methods of claims 39 to 45.

47. The use of a CEA agonist polypeptide of claim 1 for the manufacture of a medicament for the treatment of cancer.

48. The use of claim 47, wherein said polypeptide comprises the amino acid sequence of SEQ. ID NO. 1.

49. The use of an isolated, purified or recombinant nucleic acid sequence having the sequence of SEQ. ID NO. 2 for the manufacture of a medicament for the treatment of cancer.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a T L H V I K S D L V N E E A T G Q F R V -
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a D A V A F T C E P E T Q D A T Y L W W V -
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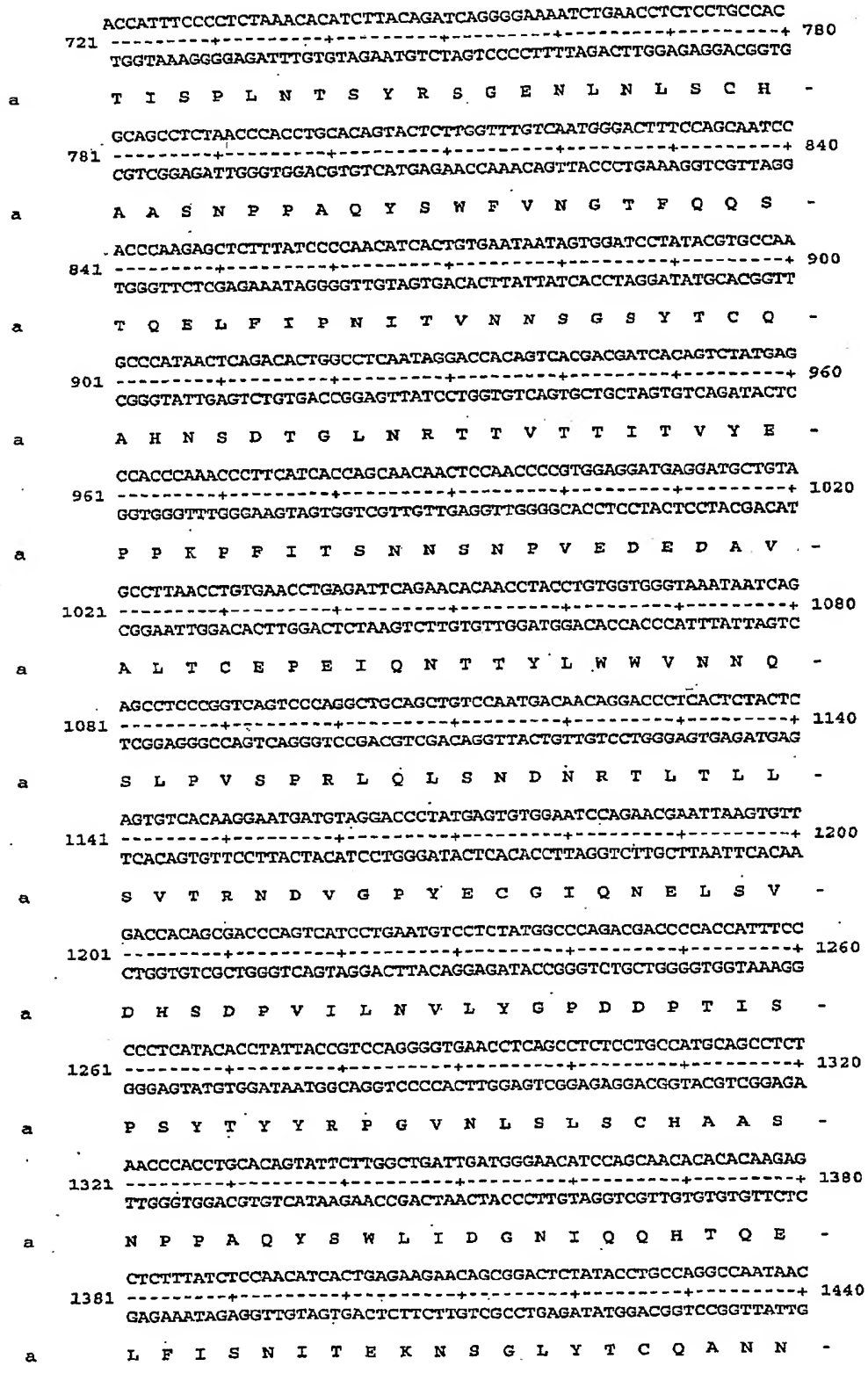
Figure 1(A)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1020
a P P K P F I T S N N S N P V E D E D A V -
GCCTTAACCTGTGAACCTGAGATTCAGAACACAACCTACCTGTGGTGGGTAAATAATCAG
1021
CGGAATTGGACACTTGGACTCTAAGTCTTGTGTTGGATGGACACCACCCATTTATTAGTC
1080
a A L T C E P E I Q N T T Y L W W V N N Q -
AGCCTCCCGGTCAGTCCCAGGCTGCAGCTGTCCAATGACAACAGGACCCTCACTCTACTC
1081
TCGGAGGGCCAGTCAGGGTCCGACGTCGACAGGTTACTGTTGTCCTGGGAGTGAGATGAG
1140
a S L P V S P R L Q L S N D N R T L T L L -
AGTGTCACAAGGAATGATGTAGGACCCTATGAGTGTGGAATCCAGAACGAATTAAGTGTT
1141
TCACAGTGTTCCTTACTACATCCTGGGATACTCACACCTTAGGTCTTGCTTAATTCACAA
1200
a S V T R N D V G P Y E C G I Q N E L S V -
GACCACAGCGACCCAGTCATCCTGAATGTCCTCTATGGCCCAGACGACCCCACCATTTCC
1201
CTGGTGTCGCTGGGTCAGTAGGACTTACAGGAGATACCGGGTCTGCTGGGGTGGTAAAGG
1260
a D H S D P V I L N V L Y G P D D P T I S -
CCCTCATACACCTATTACCGTCCAGGGGTGAACCTCAGCCTCTCCTGCCATGCAGCCTCT
1261
GGGAGTATGTGGATAATGGCAGGTCCCCACTTGGAGTCGGAGAGGACGGTACGTCGGAGA
1320
a P S Y T Y Y R P G V N L S L S C H A A S -
AACCCACCTGCACAGTATTCTTGGCTGATTGATGGGAACATCCAGCAACACACACAAGAG
1321
TTGGGTGGACGTGTCATAAGAACCGACTAACTACCCTTGTAGGTCGTTGTGTGTGTTCTC
1380
a N P P A Q Y S W L I D G N I Q Q H T Q E -
CTCTTTATCTCCAACATCACTGAGAAGAACAGCGGACTCTATACCTGCCAGGCCAATAAC
1381
GAGAAATAGAGGTTGTAGTGACTCTTCTTGTCGCCTGAGATATGGACGGTCCGGTTATTG
1440
a L F I S N I T E K N S G L Y T C Q A N N -

Figure 1(B)

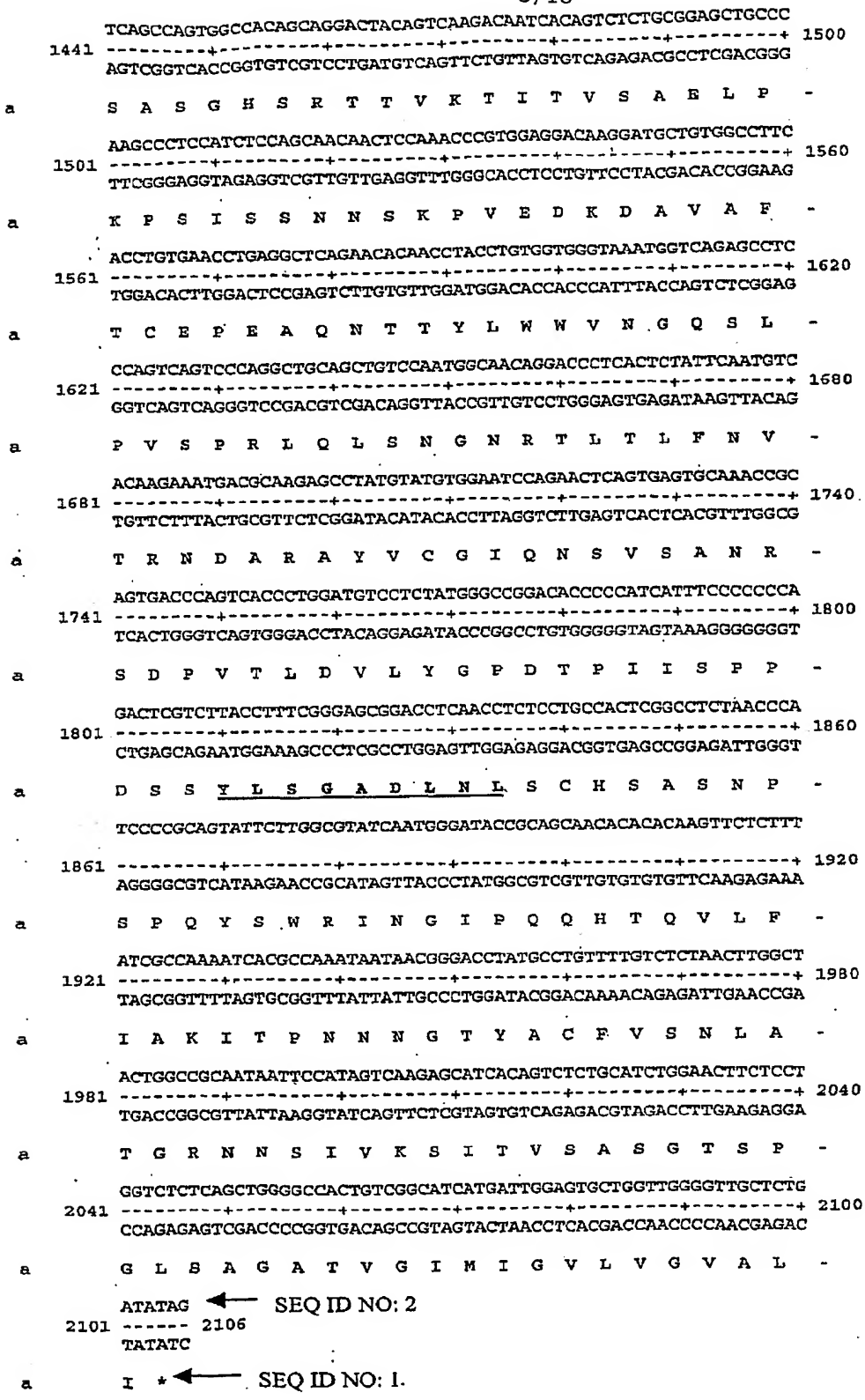
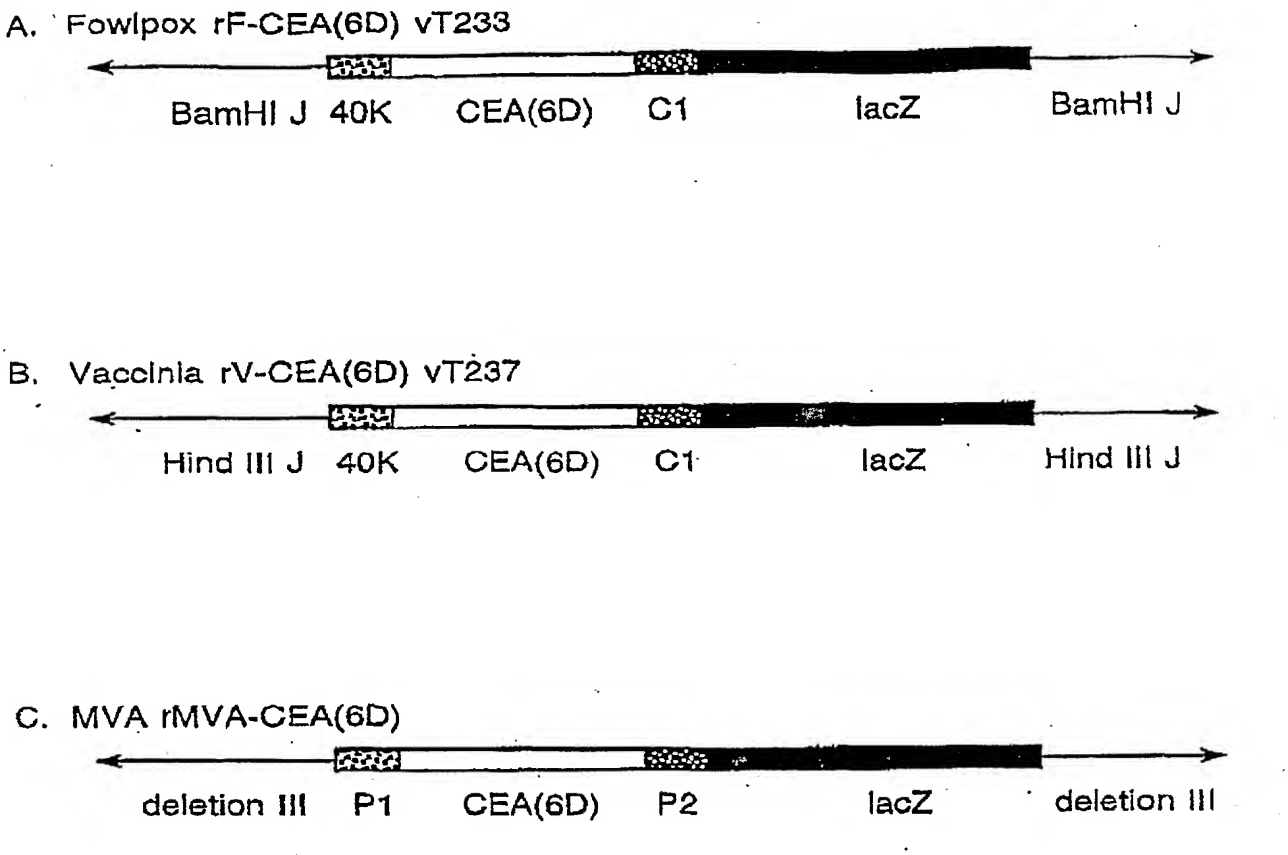

TCAGCCAGTGGCCACAGCAGGACTACAGTCAAGACAATCACAGTCTCTGCGGAGCTGCCC
1441 + 1500
AGTCGGTCACCGGTGTCGTCCTGATGTCAGTTCTGTTAGTGTCAGAGACGCCTCGACGGG
a S A S G H S R T T V K T I T V S A E L P -
AAGCCCTCCATCTCCAGCAACAACTCCAAACCCGTGGAGGACAAGGATGCTGTGGCCTTC
1501 + 1560
TTCGGGAGGTAGAGGTCGTTGTTGAGGTTTGGGCACCTCCTGTTCCTACGACACCGGAAG
a K P S I S S N N S K P V E D K D A V A F -
ACCTGTGAACCTGAGGCTCAGAACACAACCTACCTGTGGTGGGTAAATGGTCAGAGCCTC
1561 + 1620
TGGACACTTGGACTCCGAGTCTTGTGTTGGATGGACACCACCCATTTACCAGTCTCGGAG
a T C E P E A Q N T T Y L W W V N G Q S L -
CCAGTCAGTCCCAGGCTGCAGCTGTCCAATGGCAACAGGACCCTCACTCTATTCAATGTC
1621 + 1680
GGTCAGTCAGGGTCCGACGTCGACAGGTTACCGTTGTCCTGGGAGTGAGATAAGTTACAG
a P V S P R L Q L S N G N R T L T L F N V -
ACAAGAAATGACGCAAGAGCCTATGTATGTGGAATCCAGAACTCAGTGAGTGCAAACCGC
1681 + 1740
TGTTCTTTACTGCGTTCTCGGATACATACACCTTAGGTCTTGAGTCACTCACGTTTGGCG
a T R N D A R A Y V C G I Q N S V S A N R -
AGTGACCCAGTCACCCTGGATGTCCTCTATGGGCCGGACACCCCCATCATTTCCCCCCCA
1741 + 1800
TCACTGGGTCAGTGGGACCTACAGGAGATACCCGGCCTGTGGGGGTAGTAAAGGGGGGGT
a S D P V T L D V L Y G P D T P I I S P P -
GACTCGTCTTACCTTTCGGGAGCGGACCTCAACCTCTCCTGCCACTCGGCCTCTAACCCA
1801 + 1860
CTGAGCAGAATGGAAAGCCCTCGCCTGGAGTTGGAGAGGACGGTGAGCCGGAGATTGGGT
a D S S Y L S G A D L N L S C H S A S N P -
TCCCCGCAGTATTCTTGGCGTATCAATGGGATACCGCAGCAACACACACAAGTTCTCTTT
1861 + 1920
AGGGGCGTCATAAGAACCGCATAGTTACCCTATGGCGTCGTTGTGTGTGTTCAAGAGAAA
a S P Q Y S W R I N G I P Q Q H T Q V L F -
ATCGCCAAAATCACGCCAAATAATAACGGGACCTATGCCTGTTTTGTCTCTAACTTGGCT
1921 + 1980
TAGCGGTTTTAGTGCGGTTTATTATTGCCCTGGATACGGACAAAACAGAGATTGAACCGA
a I A K I T P N N N G T Y A C F V S N L A -
ACTGGCCGCAATAATTCCATAGTCAAGAGCATCACAGTCTCTGCATCTGGAACTTCTCCT
1981 + 2040
TGACCGGCGTTATTAAGGTATCAGTTCTCGTAGTGTCAGAGACGTAGACCTTGAAGAGGA
a T G R N N S I V K S I T V S A S G T S P -
GGTCTCTCAGCTGGGGCCACTGTCGGCATCATGATTGGAGTGCTGGTTGGGGTTGCTCTG
2041 + 2100
CCAGAGAGTCGACCCCGGTGACAGCCGTAGTACTAACCTCACGACCAACCCCAACGAGAC
a G L S A G A T V G I M I G V L V G V A L -
ATATAG ← SEQ ID NO: 2
2101 ------ 2106
TATATC
a I * ← SEQ ID NO: 1.

Figure 1(C)

Genomic Structure of Recombinant Poxviruses Expressing CEA(6D)



BamHI J is the site of insertion in the fowlpox genome of the foreign genes.
Hind III J is the site of insertion in the vaccinia virus genome.
Deletion III is the site of insertion in the MVA genome.
40K, C1, P1 and P2 are poxviral promoters.

(not drawn to scale)

Figure 2

ALVAC(2)-CEAmod/hB7.1 (vCP1586)

(ALVAC *Xho*I Restriction Map)

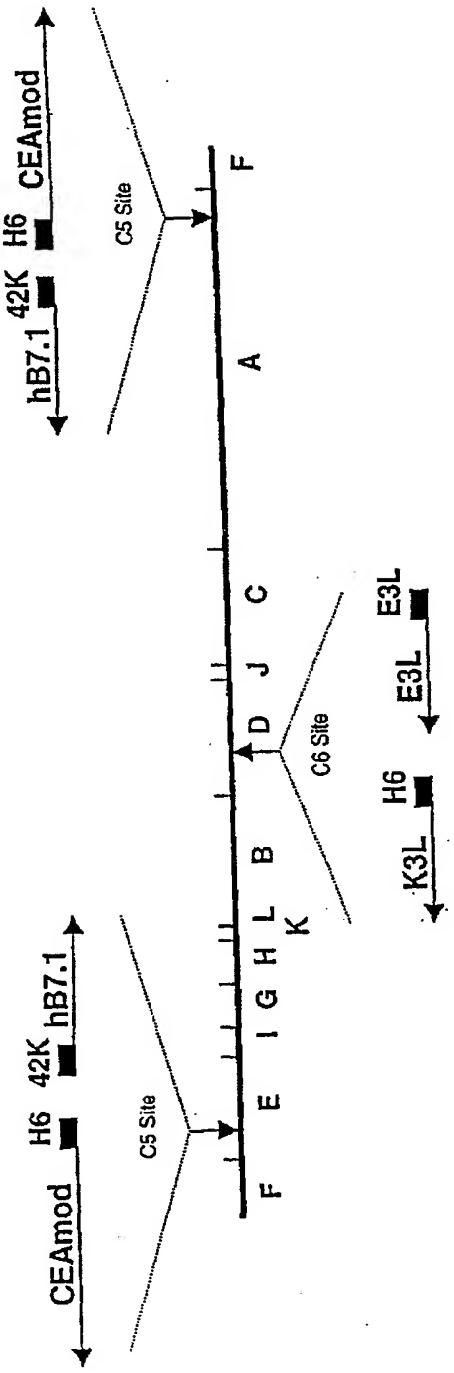


Figure 3

Sequence of the H6-promoted human CEAmod/42K-promoted B7.1 insertion cassette

```
        10          20          30          40          50          60
GCCCTTTCGT CTCGCGCGTT TCGGTGATGA CGGTGAAAAC CTCTGACACA TGCAGCTCCC
CGGGAAAGCA GAGCGCGCAA AGCCACTACT GCCACTTTTG GAGACTGTGT ACGTCGAGGG

        70          80          90          100         110         120
GGAGACGGTC ACAGCTTGTC TGTAAGCGGA TGCCGGGAGC AGACAAGCCC GTCAGGGCGC
CCTCTGCCAG TGTCGAACAG ACATTCGCCT ACGGCCCTCG TCTGTTCGGG CAGTCCCGCG

        130         140         150         160         170         180
GTCAGCGGGT GTTGGCGGGT GTCGGGGCTG GCTTAACTAT GCGGCATCAG AGCAGATTGT
CAGTCGCCCA CAACCGCCCA CAGCCCCGAC CGAATTGATA CGCCGTAGTC TCGTCTAACA

        190         200         210         220         230         240
ACTGAGAGTG CACCATATGC GGTGTGAAAT ACCGCACAGA TGCGTAAGGA GAAAATACCG
TGACTCTCAC GTGGTATACG CCACACTTTA TGGCGTGTCT ACGCATTCCT CTTTTATGGC

        250         260         270         280         290         300
CATCAGGCGC CATTCGCCAT TCAGGCTGCG CAACTGTTGG GAAGGGCGAT CGGTGCGGGC
GTAGTCCGCG GTAAGCGGTA AGTCCGACGC GTTGACAACC CTTCCCGCTA GCCACGCCCG

        310         320         330         340         350         360
CTCTTCGCTA TTACGCCAGC TGGCGAAAGG GGGATGTGCT GCAAGGCGAT TAAGTTGGGT
GAGAAGCGAT AATGCGGTCG ACCGCTTTCC CCCTACACGA CGTTCCGCTA ATTCAACCCA

        370         380         390         400         410         420
AACGCCAGGG TTTTCCCAGT CACGACGTTG TAAAACGACG GCCAGTGCCA AGCTTGGCTG
TTGCGGTCCC AAAAGGGTCA GTGCTGCAAC ATTTTGCTGC CGGTCACGGT TCGAACCGAC

        430         440         450         460         470         480
CAGGTATTCT AAACTAGGAA TAGATGAAAT TATGTGCAAA GGAGATACCT TTAGATATGG
GTCCATAAGA TTTGATCCTT ATCTACTTTA ATACACGTTT CCTCTATGGA AATCTATACC
  _________c_____ALVAC'S C5 LOCUS LEFT FLANKING ARM____c__________>

        490         500         510         520         530         540
ATCTGATTTA TTTGGTTTTT CATAATCATA ATCTAACAAC ATTTTCACTA TACTATACCT
TAGACTAAAT AAACCAAAAA GTATTAGTAT TAGATTGTTG TAAAAGTGAT ATGATATGGA
__________c____ALVAC'S C5 LOCUS LEFT FLANKING ARM_____c__________>

        550         560         570         580         590         600
TCTTGCACAA GTCGCCATTA GTAGTATAGA CTTATACTTT GTAACCATAG TATACTTTAG
AGAACGTGTT CAGCGGTAAT CATCATATCT GAATATGAAA CATTGGTATC ATATGAAATC
__________c____ALVAC'S C5 LOCUS LEFT FLANKING ARM_____c__________>

        610         620         630         640         650         660
CGCGTCATCT TCTTCATCTA AAACAGATTT ACAACAATAA TCATCGTCGT CATCTTCATC
GCGCAGTAGA AGAAGTAGAT TTTGTCTAAA TGTTGTTATT AGTAGCAGCA GTAGAAGTAG
__________c____ALVAC'S C5 LOCUS LEFT FLANKING ARM_____c__________>

        670         680         690         700         710         720
TTCATTAAAG TTTTCATATT CAATAACTTT CTTTTCTAAA ACATCATCTG AATCAATAAA
AAGTAATTTC AAAAGTATAA GTTATTGAAA GAAAAGATTT TGTAGTAGAC TTAGTTATTT
__________c____ALVAC'S C5 LOCUS LEFT FLANKING ARM_____c__________>

        730         740         750         760         770         780
CATAGAACGG TATAGAGCGT TAATCTCCAT TGTAAAATAT ACTAACGCGT TGCTCATGAT
```

Figure 4(A)

Sequence of the H6-promoted human CEAmod/42K-promoted B7.1 insertion cassette (Cont.)

```
GTATCTTGCC ATATCTCGCA ATTAGAGGTA ACATTTTATA TGATTGCGCA ACGAGTACTA
_________c____ALVAC'S C5 LOCUS LEFT FLANKING ARM_____c_________>

      790        800        810        820        830        840
GTACTTTTTT TCATTATTTA GAAATTATGC ATTTTAGATC TTTATAAGCG GCCGTGATTA
CATGAAAAAA AGTAATAAAT CTTTAATACG TAAAATCTAG AAATATTCGC CGGCACTAAT
________ALVAC'S C5 LOCUS LEFT FLANKING ARM_c_______>

      850        860        870        880        890        900
ACTAGTCATA AAAACCCGGG ATCGATTCTA GACTCGACCT CGAAGCTTGC ATGCCGGTCG
TGATCAGTAT TTTTGGGCCC TAGCTAAGAT CTGAGCTGGA GCTTCGAACG TACGGCCAGC

      910        920        930        940        950        960
AGCTATATCA GAGCAACCCC AACCAGCACT CCAATCATGA TGCCGACAGT GGCCCCAGCT
TCGATATAGT CTCGTTGGGG TTGGTCGTGA GGTTAGTACT ACGGCTGTCA CCGGGGTCGA
 <________a_________a__MODIFIED CEA ORF.__a_________a_________

      970        980        990       1000       1010       1020
GAGAGACCAG GAGAAGTTCC AGATGCAGAG ACTGTGATGC TCTTGACTAT GGAATTATTG
CTCTCTGGTC CTCTTCAAGG TCTACGTCTC TGACACTACG AGAACTGATA CCTTAATAAC
<_________a_________a_MODIFIED CEA ORF.___a_________a_________

      1030       1040       1050       1060       1070       1080
CGGCCAGTAG CCAAGTTAGA GACAAAACAG GCATAGGTCC CGTTATTATT TGGCGTGATT
GCCGGTCATC GGTTCAATCT CTGTTTTGTC CGTATCCAGG GCAATAATAA ACCGCACTAA
<_________a_________a_MODIFIED CEA ORF.___a_________a_________

      1090       1100       1110       1120       1130       1140
TTGGCGATAA AGAGAACTTG TGTGTGTTGC TGCGGTATCC CATTGATACG CCAAGAATAC
AACCGCTATT TCTCTTGAAC ACACACAACG ACGCCATAGG GTAACTATGC GGTTCTTATG
<_________a_________a_MODIFIED CEA ORF.___a_________a_________

      1150       1160       1170       1180       1190       1200
TGCGGGGATG GGTTAGAGGC CGAGTGGCAG GAGAGGTTGA GGTCCGCTCC CGAAAGGTAA
ACGCCCCTAC CCAATCTCCG GCTCACCGTC CTCTCCAACT CCAGGCGAGG GCTTTCCATT
<_________a_________a_MODIFIED CEA ORF.___a_________a_________

      1210       1220       1230       1240       1250       1260
GACGAGTCTG GGGGGGAAAT GATGGGGGTG TCCGGCCCAT AGAGGACATC CAGGGTGACT
CTGCTCAGAC CCCCCCTTTA CTACCCCCAC AGGCCGGGTA TCTCCTGTAG GTCCCACTGA
<_________a_________a_MODIFIED CEA ORF.___a_________a_________

      1270       1280       1290       1300       1310       1320
GGGTCACTGC GGTTTGCACT CACTGAGTTC TGGATTCCAC ATACATAGGC TCTTGCGTCA
CCCAGTGACG CCAAACGTGA GTGACTCAAG ACCTAAGGTG TATGTATCCG AGAACGCAGT
<_________a_________a_MODIFIED CEA ORF.___a_________a_________

      1330       1340       1350       1360       1370       1380
TTTCTTGTGA CATTGAATAG AGTGAGGGTC CTGTTGCCAT TGGACAGCTG CAGCCTGGGA
AAAGAACACT GTAACTTATC TCACTCCCAG GACAACGGTA ACCTGTCGAC GTCGGACCCT
<_________a_________a_MODIFIED CEA ORF.___a_________a_________

      1390       1400       1410       1420       1430       1440
CTGACTGGGA GGCTCTGACC ATTTACCCAC CACAGGTAGG TTGTGTTCTG AGCCTCAGGT
GACTGACCCT CCGAGACTGG TAAATGGGTG GTGTCCATCC AACACAAGAC TCGGAGTCCA
<_________a_____.....____a_MODIFIED CEA ORF.___a_________a_____.....
```

Figure 4(B)

Sequence of the H6-promoted human CEAmod/42K-promoted B7.1 insertion cassette (Cont.)

```
      1450       1460       1470       1480       1490       1500
TCACAGGTGA AGGCCACAGC ATCCTTGTCC TCCACGGGTT TGGAGTTGTT GCTGGAGATG
AGTGTCCACT TCCGGTGTCG TAGGAACAGG AGGTGCCCAA ACCTCAACAA CGACCTCTAC
<__________a__________a_MODIFIED CEA ORF.___a__________a__________

      1510       1520       1530       1540       1550       1560
GAGGGCTTGG GCAGCTCCGC AGAGACTGTG ATTGTCTTGA CTGTAGTCCT GCTGTGGCCA
CTCCCGAACC CGTCGAGGCG TCTCTGACAC TAACAGAACT GACATCAGGA CGACACCGGT
<__________a__________a_MODIFIED CEA ORF.___a__________a__________

      1570       1580       1590       1600       1610       1620
CTGGCTGAGT TATTGGCCTG GCAGGTATAG AGTCCGCTGT TCTTCTCAGT GATGTTGGAG
GACCGACTCA ATAACCGGAC CGTCCATATC TCAGGCGACA AGAAGAGTCA CTACAACCTC
<__________a__________a_MODIFIED CEA ORF.___a__________a__________

      1630       1640       1650       1660       1670       1680
ATAAAGAGCT CTTGTGTGTG TTGCTGGATG TTCCCATCAA TCAGCCAAGA ATACTGTGCA
TATTTCTCGA GAACACACAC AACGACCTAC AAGGGTAGTT AGTCGGTTCT TATGACACGT
<__________a__________a_MODIFIED CEA ORF.___a__________a__________

      1690       1700       1710       1720       1730       1740
GGTGGGTTAG AGGCTGCATG GCAGGAGAGG CTGAGGTTCA CCCCTGGACG GTAATAGGTG
CCACCCAATC TCCGACGTAC CGTCCTCTCC GACTCCAAGT GGGGACCTGC CATTATCCAC
<__________a__________a_MODIFIED CEA ORF.___a__________a__________

      1750       1760       1770       1780       1790       1800
TATGAGGGGG AAATGGTGGG GTCGTCTGGG CCATAGAGGA CATTCAGGAT GACTGGGTCG
ATACTCCCCC TTTACCACCC CAGCAGACCC GGTATCTCCT GTAAGTCCTA CTGACCCAGC
<__________a__________a_MODIFIED CEA ORF.___a__________a__________

      1810       1820       1830       1840       1850       1860
CTGTGGTCAA CACTTAATTC GTTCTGGATT CCACACTCAT AGGGTCCTAC ATCATTCCTT
GACACCAGTT GTGAATTAAG CAAGACCTAA GGTGTGAGTA TCCCAGGATG TAGTAAGGAA
<__________a__________a_MODIFIED CEA ORF.___a__________a__________

      1870       1880       1890       1900       1910       1920
GTGACACTGA GTAGAGTGAG GGTCCTGTTG TCATTGGACA GCTGCAGCCT GGGACTGACC
CACTGTGACT CATCTCACTC CCAGGACAAC AGTAACCTGT CGACGTCGGA CCCTGACTGG
<__________a__________a_MODIFIED CEA ORF.___a__________a__________

      1930       1940       1950       1960       1970       1980
GGGAGGCTCT GATTATTTAC CCACCACAGG TAGGTTGTGT TCTGAATCTC AGGTTCACAG
CCCTCCGAGA CTAATAAATG GGTGGTGTCC ATCCAACACA AGACTTAGAG TCCAAGTGTC
<__________a__________a_MODIFIED CEA ORF.___a__________a__________

      1990       2000       2010       2020       2030       2040
GTTAAGGCTA CAGCATCCTC ATCCTCCACG GGGTTGGAGT TGTTGCTGGT GATGAAGGGT
CAATTCCGAT GTCGTAGGAG TAGGAGGTGC CCCAACCTCA ACAACGACCA CTACTTCCCA
<__________a__________a_MODIFIED CEA ORF.___a__________a__________

      2050       2060       2070       2080       2090       2100
TTGGGTGGCT CATAGACTGT GATCGTCGTG ACTGTGGTCC TATTGAGGCC AGTGTCTGAG
AACCCACCGA GTATCTGACA CTAGCAGCAC TGACACCAGG ATAACTCCGG TCACAGACTC
<__________a__________a_MODIFIED CEA ORF.___a__________a__________

      2110       2120       2130       2140       2150       2160
TTATGGGCTT GGCACGTATA GGATCCACTA TTATTCACAG TGATGTTGGG GATAAAGAGC
```

Figure 4(C)

Sequence of the H6-promoted human CEAmod/42K-promoted B7.1 insertion cassette (Cont.)

```
 AATACCCGAA CCGTGCATAT CCTAGGTGAT AATAAGTGTC ACTACAACCC CTATTTCTCG
<_____a_____a_MODIFIED CEA ORF._____a_____a_____

      2170       2180       2190       2200       2210       2220
 TCTTGGGTGG ATTGCTGGAA AGTCCCATTG ACAAACCAAG AGTACTGTGC AGGTGGGTTA
 AGAACCCACC TAACGACCTT TCAGGGTAAC TGTTTGGTTC TCATGACACG TCCACCCAAT
<_____a_____a_MODIFIED CEA ORF._____a_____a_____

      2230       2240       2250       2260       2270       2280
 GAGGCTGCGT GGCAGGAGAG GTTCAGATTT TCCCCTGATC TGTAAGATGT GTTTAGAGGG
 CTCCGACGCA CCGTCCTCTC CAAGTCTAAA AGGGGACTAG ACATTCTACA CAAATCTCCC
<_____a_____a_MODIFIED CEA ORF._____a_____a_____

      2290       2300       2310       2320       2330       2340
 GAAATGGTGG GGGCATCCGG GCCATAGAGG ACATTCAGGA TGACTGAATC ACTGCGCCTG
 CTTTACCACC CCCGTAGGCC CGGTATCTCC TGTAAGTCCT ACTGACTTAG TGACGCGGAC
<_____a_____a_MODIFIED CEA ORF._____a_____a_____

      2350       2360       2370       2380       2390       2400
 GCACTCACTG GGTTCTGGGT TTCACATTTG TAGCTTGCTG TGTCATTTCT TGTGACATTG
 CGTGAGTGAC CCAAGACCCA AAGTGTAAAC ATCGAACGAC ACAGTAAAGA ACACTGTAAC
<_____a_____a_MODIFIED CEA ORF._____a_____a_____

      2410       2420       2430       2440       2450       2460
 AATAGAGTGA GGGTCCTGTT GCCATTGGAC AGCTGCAGCC TGGGACTGAC CGGGAGGCTC
 TTATCTCACT CCCAGGACAA CGGTAACCTG TCGACGTCGG ACCCTGACTG GCCCTCCGAG
<_____a_____a_MODIFIED CEA ORF._____a_____a_____

      2470       2480       2490       2500       2510       2520
 TGATTGTTTA CCCACCACAG GTAGGTTGCG TCCTGAGTCT CAGGTTCACA GGTGAAGGCC
 ACTAACAAAT GGGTGGTGTC CATCCAACGC AGGACTCAGA GTCCAAGTGT CCACTTCCGG
<_____a_____a_MODIFIED CEA ORF._____a_____a_____

      2530       2540       2550       2560       2570       2580
 ACAGCATCCT TGTCCTCCAC GGGTTTGGAG TTGTTGCTGG AGATGGAGGG CTTGGGCAGC
 TGTCGTAGGA ACAGGAGGTG CCCAAACCTC AACAACGACC TCTACCTCCC GAACCCGTCG
<_____a_____a_MODIFIED CEA ORF._____a_____a_____

      2590       2600       2610       2620       2630       2640
 TCCGGGTATA CCCGGAACTG GCCAGTTGCT TCTTCATTCA CAAGATCTGA CTTTATGACG
 AGGCCCATAT GGGCCTTGAC CGGTCAACGA AGAAGTAAGT GTTCTAGACT GAAATACTGC
<_____a_____a_MODIFIED CEA ORF._____a_____a_____

      2650       2660       2670       2680       2690       2700
 TGTAGGGTGT AGAATCCTGT GTCATTCTGG ATGATGTTCT GGATCAGCAG GGATGCATTG
 ACATCCCACA TCTTAGGACA CAGTAAGACC TACTACAAGA CCTAGTCGTC CCTACGTAAC
<_____a_____a_MODIFIED CEA ORF._____a_____a_____

      2710       2720       2730       2740       2750       2760
 GGGTATATTA TCTCTCGACC ACTGTATGCG GGCCCTGGGG TAGCTTGTTG AGTTCCTATT
 CCCATATAAT AGAGAGCTGG TGACATACGC CCGGGACCCC ATCGAACAAC TCAAGGATAA
<_____a_____a_MODIFIED CEA ORF._____a_____a_____

      2770       2780       2790       2800       2810       2820
 ACATATCCTA TAATTTGACG GTTGCCATCC ACTCTTTCAC CTTTGTACCA GCTGTAGCCA
 TGTATAGGAT ATTAAACTGC CAACGGTAGG TGAGAAAGTG GAAACATGGT CGACATCGGT
<_____a_____a_MODIFIED CEA ORF._____a_____a_____
```

Figure 4(D)

Sequence of the H6-promoted human CEAmod/42K-promoted B7.1 insertion cassette (Cont.)

```
      2830       2840       2850       2860       2870       2880
AAAAGATGCT GGGGCAGATT GTGGACAAGT AGAAGCACCT CCTTCCCCTC TGCGACATTG
TTTTCTACGA CCCCGTCTAA CACCTGTTCA TCTTCGTGGA GGAAGGGGAG ACGCTGTAAC
<__________a__________a_MODIFIED CEA ORF.___a__________a__________

      2890       2900       2910       2920       2930       2940
AACGGCGTGG ATTCAATAGT GAGCTTGGCA GTGGTGGGCG GGTTCCAGAA GGTTAGAAGT
TTGCCGCACC TAAGTTATCA CTCGAACCGT CACCACCCGC CCAAGGTCTT CCAATCTTCA
<__________a__________a_MODIFIED CEA ORF.___a__________a__________

      2950       2960       2970       2980       2990       3000
GAGGCTGTGA GCAGGAGCCT CTGCCAGGGG ATGCACCATC TGTGGGGAGG GGCCGAGGGA
CTCCGACACT CGTCCTCGGA GACGGTCCCC TACGTGGTAG ACACCCCTCC CCGGCTCCCT
<__________a__________a_MODIFIED CEA ORF.___a__________a__________

      3010       3020       3030       3040       3050       3060
GACTCCATTA CGATACAAAC TTAACGGATA TCGCGATAAT GAAATAATTT ATGATTATTT
CTGAGGTAAT GCTATGTTTG AATTGCCTAT AGCGCTATTA CTTTATTAAA TACTAATAAA
<_________
          __e__________e___VACCINIA'S H6 PROMOTER_________e__________>

      3070       3080       3090       3100       3110       3120
CTCGCTTTCA ATTTAACACA ACCCTCAAGA ACCTTTGTAT TTATTTTCAC TTTTTAAGTA
GAGCGAAAGT TAAATTGTGT TGGGAGTTCT TGGAAACATA AATAAAAGTG AAAAATTCAT
___________e__________VACCINIA'S H6 PROMOTER___________e__________>

      3130       3140       3150       3160       3170       3180
TAGAATAAAG AAGCTCTAAT TAATTAACGA GCAGATAGTC TCGTTCTCGC CCTGCCTGAT
ATCTTATTTC TTCGAGATTA ATTAATTGCT CGTCTATCAG AGCAAGAGCG GGACGGACTA
___________e__>

      3190       3200       3210       3220       3230       3240
GACTAATTAA TTAACCCGGA TCCGAATTCT TTATTGGGAA GAATATGATA ATATTTTGGG
CTGATTAATT AATTGGGCCT AGGCTTAAGA AATAACCCTT CTTATACTAT TATAAAACCC
                                _f_________42K PROMOTER_f__________>

      3250       3260       3270       3280       3290       3300
ATTTCAAAAT TGAAAATATA TAATTACAAT ATAAAATGGG CCACACACGG AGGCAGGGAA
TAAAGTTTTA ACTTTTATAT ATTAATGTTA TATTTTACCC GGTGTGTGCC TCCGTCCCTT
                                      _______HUMAN B7.1 ORF________>
___________f_42K PROMOTER_________f_____>

      3310       3320       3330       3340       3350       3360
CATCACCATC CAAGTGTCCA TACCTCAATT TCTTTCAGCT CTTGGTGCTG GCTGGTCTTT
GTAGTGGTAG GTTCACAGGT ATGGAGTTAA AGAAAGTCGA GAACCACGAC CGACCAGAAA
___________b__________b___HUMAN B7.1 ORF_____b__________b__________>

      3370       3380       3390       3400       3410       3420
CTCACTTCTG TTCAGGTGTT ATCCACGTGA CCAAGGAAGT GAAAGAAGTG GCAACGCTGT
GAGTGAAGAC AAGTCCACAA TAGGTGCACT GGTTCCTTCA CTTTCTTCAC CGTTGCGACA
___________b__________b___HUMAN B7.1 ORF_____b__________b__________>

      3430       3440       3450       3460       3470       3480
CCTGTGGTCA CAATGTTTCT GTTGAAGAGC TGGCACAAAC TCGCATCTAC TGGCAAAAGG
GGACACCAGT GTTACAAAGA CAACTTCTCG ACCGTGTTTG AGCGTAGATG ACCGTTTTCC
___________b__________b___HUMAN B7.1 ORF_____b__________b__________>
```

Figure 4(E)

Sequence of the H6-promoted human CEAmod/42K-promoted B7.1 insertion cassette (Cont.)

```
      3490        3500        3510        3520        3530        3540
AGAAGAAAAT GGTGCTGACT ATGATGTCTG GAGACATGAA TATATGGCCC GAGTACAAGA
TCTTCTTTTA CCACGACTGA TACTACAGAC CTCTGTACTT ATATACCGGG CTCATGTTCT
__________b__________b___HUMAN B7.1 ORF____b__________b__________>

      3550        3560        3570        3580        3590        3600
ACCGGACCAT CTTTGATATC ACTAATAACC TCTCCATTGT GATCCTGGCT CTGCGCCCAT
TGGCCTGGTA GAAACTATAG TGATTATTGG AGAGGTAACA CTAGGACCGA GACGCGGGTA
__________b__________b___HUMAN B7.1 ORF____b__________b__________>

      3610        3620        3630        3640        3650        3660
CTGACGAGGG CACATACGAG TGTGTTGTTC TGAAGTATGA AAAAGACGCT TTCAAGCGGG
GACTGCTCCC GTGTATGCTC ACACAACAAG ACTTCATACT TTTTCTGCGA AAGTTCGCCC
__________b__________b___HUMAN B7.1 ORF____b__________b__________>

      3670        3680        3690        3700        3710        3720
AACACCTGGC TGAAGTGACG TTATCAGTCA AAGCTGACTT CCCTACACCT AGTATATCTG
TTGTGGACCG ACTTCACTGC AATAGTCAGT TTCGACTGAA GGGATGTGGA TCATATAGAC
__________b__________b___HUMAN B7.1 ORF____b__________b__________>

      3730        3740        3750        3760        3770        3780
ACTTTGAAAT TCCAACTTCT AATATTAGAA GGATAATTTG CTCAACCTCT GGAGGTTTTC
TGAAACTTTA AGGTTGAAGA TTATAATCTT CCTATTAAAC GAGTTGGAGA CCTCCAAAAG
__________b__________b___HUMAN B7.1 ORF____b__________b__________>

      3790        3800        3810        3820        3830        3840
CAGAGCCTCA CCTCTCCTGG TTGGAAAATG GAGAAGAATT AAATGCCATC AACACAACAG
GTCTCGGAGT GGAGAGGACC AACCTTTTAC CTCTTCTTAA TTTACGGTAG TTGTGTTGTC
__________b__________b___HUMAN B7.1 ORF____b__________b__________>

      3850        3860        3870        3880        3890        3900
TTTCCCAAGA TCCTGAAACT GAGCTCTATG CTGTTAGCAG CAAACTGGAT TTCAATATGA
AAAGGGTTCT AGGACTTTGA CTCGAGATAC GACAATCGTC GTTTGACCTA AAGTTATACT
__________b__________b___HUMAN B7.1 ORF____b__________b__________>

      3910        3920        3930        3940        3950        3960
CAACCAACCA CAGCTTCATG TGTCTCATCA AGTATGGACA TTTAAGAGTG AATCAGACCT
GTTGGTTGGT GTCGAAGTAC ACAGAGTAGT TCATACCTGT AAATTCTCAC TTAGTCTGGA
__________b__________b___HUMAN B7.1 ORF____b__________b__________>

      3970        3980        3990        4000        4010        4020
TCAACTGGAA TACAACCAAG CAAGAGCATT TTCCTGATAA CCTGCTCCCA TCCTGGGCCA
AGTTGACCTT ATGTTGGTTC GTTCTCGTAA AAGGACTATT GGACGAGGGT AGGACCCGGT
__________b__________b___HUMAN B7.1 ORF____b__________b__________>

      4030        4040        4050        4060        4070        4080
TTACCTTAAT CTCAGTAAAT GGAATTTTCG TGATATGCTG CCTGACCTAC TGCTTTGCCC
AATGGAATTA GAGTCATTTA CCTTAAAAGC ACTATACGAC GGACTGGATG ACGAAACGGG
__________b__________b___HUMAN B7.1 ORF____b__________b__________>

      4090        4100        4110        4120        4130        4140
CACGCTGCAG AGAGAGAAGG AGGAATGAGA GATTGAGAAG GGAAAGTGTA CGCCCTGTAT
GTGCGACGTC TCTCTCTTCC TCCTTACTCT CTAACTCTTC CCTTTCACAT GCGGGACATA
__________b__________b___HUMAN B7.1 ORF____b__________b__________>

      4150        4160        4170        4180        4190        4200
```

Figure 4(F)

Sequence of the H6-promoted human CEAmod/42K-promoted B7.1 insertion cassette (Cont.)

```
AAGTCGACGA TCCTTTTTAT AGCTAATTAG TCACGTACCT TTGAGAGTAC CACTTCAGCT
TTCAGCTGCT AGGAAAAATA TCGATTAATC AGTGCATGGA AACTCTCATG GTGAAGTCGA
__>
                                                    ___ALVAC'S C5 ____>

      4210        4220        4230        4240        4250        4260
ACCTCTTTTG TGTCTCAGAG TAACTTTCTT TAATCAATTC CAAAACAGTA TATGATTTTC
TGGAGAAAAC ACAGAGTCTC ATTGAAAGAA ATTAGTTAAG GTTTTGTCAT ATACTAAAAG
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      4270        4280        4290        4300        4310        4320
CATTTCTTTC AAAGATGTAG TTTACATCTG CTCCTTTGTT GAAAAGTAGC CTGAGCACTT
GTAAAGAAAG TTTCTACATC AAATGTAGAC GAGGAAACAA CTTTTCATCG GACTCGTGAA
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      4330        4340        4350        4360        4370        4380
CTTTTCTACC ATGAATTACA GCTGGCAAGA TCAATTTTTC CCAGTTCTGG ACATTTTATT
GAAAAGATGG TACTTAATGT CGACCGTTCT AGTTAAAAAG GGTCAAGACC TGTAAAATAA
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      4390        4400        4410        4420        4430        4440
TTTTTTAAGT AGTGTGCTAC ATATTTCAAT ATTTCCAGAT TGTACAGCGA TCATTAAAGG
AAAAAATTCA TCACACGATG TATAAAGTTA TAAAGGTCTA ACATGTCGCT AGTAATTTCC
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      4450        4460        4470        4480        4490        4500
AGTACGTCCC ATGTTATCCA GCAAGTCAGT ATCAGCACCT TTGTTCAATA GAAGTTTAAC
TCATGCAGGG TACAATAGGT CGTTCAGTCA TAGTCGTGGA AACAAGTTAT CTTCAAATTG
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      4510        4520        4530        4540        4550        4560
CATTGTTAAA TTTTTATTTG ATACGGCTAT ATGTAGAGGA GTTAACCGAT CCGTGTTTGA
GTAACAATTT AAAAATAAAC TATGCCGATA TACATCTCCT CAATTGGCTA GGCACAAACT
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      4570        4580        4590        4600        4610        4620
AATATCTACA TCCGCCGAAT GAGCCAATAG AAGTTTAACC AAATTAACTT TGTTAAGGTA
TTATAGATGT AGGCGGCTTA CTCGGTTATC TTCAAATTGG TTTAATTGAA ACAATTCCAT
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      4630        4640        4650        4660        4670        4680
AGCTGCCAAA CACAAAGGAG TAAAGCCTCC GCTGTAAAGA ACATTGTTTA CATAGTTATT
TCGACGGTTT GTGTTTCCTC ATTTCGGAGG CGACATTTCT TGTAACAAAT GTATCAATAA
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      4690        4700        4710        4720        4730        4740
CTTCAACAGA TCTTTCACTA TTTTGTAGTC GTCTCTCAAC ACCGCATCAT GCAGACAAGA
GAAGTTGTCT AGAAAGTGAT AAAACATCAG CAGAGAGTTG TGGCGTAGTA CGTCTGTTCT
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      4750        4760        4770        4780        4790        4800
AGTTGTGCAT TCAGTAACTA CAGGTTTAGC TCCATACCTC ATCAAGATTT TTATAGCCTC
TCAACACGTA AGTCATTGAT GTCCAAATCG AGGTATGGAG TAGTTCTAAA AATATCGGAG
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      4810        4820        4830        4840        4850        4860
GGTATTCTTG AACATTACAG CCATTTCAAG AGGAGATTGT AGAGTACCAT ATTCCGTGTT
```

Figure 4(G)

Sequence of the H6-promoted human CEAmod/42K-promoted B7.1 insertion cassette (Cont.)

```
CCATAAGAAC TTGTAATGTC GGTAAAGTTC TCCTCTAACA TCTCATGGTA TAAGGCACAA
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      4870       4880       4890       4900       4910       4920
AGGGTCGAAT CCATTGTCCA AAAACCTATT TAGAGATGCA TTGTCATTAT CCATGATAGC
TCCCAGCTTA GGTAACAGGT TTTTGGATAA ATCTCTACGT AACAGTAATA GGTACTATCG
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      4930       4940       4950       4960       4970       4980
CTCACAGACG TATATGTAAG CCATCTTGAA TGTATAATTT TGTTGTTTTC AACAACCGCT
GAGTGTCTGC ATATACATTC GGTAGAACTT ACATATTAAA ACAACAAAAG TTGTTGGCGA
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      4990       5000       5010       5020       5030       5040
CGTGAACAGC TTCTATACTT TTTCATTTTC TTCATGATTA ATATAGTTTA CGGAATATAA
GCACTTGTCG AAGATATGAA AAAGTAAAAG AAGTACTAAT TATATCAAAT GCCTTATATT
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      5050       5060       5070       5080       5090       5100
GTATACAAAA AGTTTATAGT AATCTCATAA TATCTGAAAC ACATACATAA AACATGGAAG
CATATGTTTT TCAAATATCA TTAGAGTATT ATAGACTTTG TGTATGTATT TTGTACCTTC
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      5110       5120       5130       5140       5150       5160
AATTACACGA TGTCGTTGAG ATAAATGGCT TTTTATTGTC ATAGTTTACA AATTCGCAGT
TTAATGTGCT ACAGCAACTC TATTTACCGA AAAATAACAG TATCAAATGT TTAAGCGTCA
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      5170       5180       5190       5200       5210       5220
AATCTTCATC TTTTACGAAT ATTGCAGAAT CTGTTTTATC CAACCAGTGA TTTTTGTATA
TTAGAAGTAG AAAATGCTTA TAACGTCTTA GACAAAATAG GTTGGTCACT AAAAACATAT
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      5230       5240       5250       5260       5270       5280
ATATAACTGG TATCCTATCT TCCGATAGAA TGCTGTTATT TAACATTTTT GCACCTATTA
TATATTGACC ATAGGATAGA AGGCTATCTT ACGACAATAA ATTGTAAAAA CGTGGATAAT
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      5290       5300       5310       5320       5330       5340
AGTTACATCT GTCAAATCCA TCTTTCCAAC TGACTTTATG TAACGATGCG AAATAGCATT
TCAATGTAGA CAGTTTAGGT AGAAAGGTTG ACTGAAATAC ATTGCTACGC TTTATCGTAA
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      5350       5360       5370       5380       5390       5400
TATCACTATG TCGTACCCAA TTATCATGAC AAGATTCTCT TAAATACGTA ATCTTATTAT
ATAGTGATAC AGCATGGGTT AATAGTACTG TTCTAAGAGA ATTTATGCAT TAGAATAATA
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      5410       5420       5430       5440       5450       5460
CTCTTGCATA TTCGTAATAG TAATTGTAAA GAGTATACGA TAACAGTATA GATATACACG
GAGAACGTAT AAGCATTATC ATTAACATTT CTCATATGCT ATTGTCATAT CTATATGTGC
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      5470       5480       5490       5500       5510       5520
TGATATAAAT ATTTAACCCC ATTCCTGAGT AAAATAATTA CGATATTACA TTTCCTTTTA
ACTATATTTA TAAATTGGGG TAAGGACTCA TTTTATTAAT GCTATAATGT AAAGGAAAAT
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>
```

Figure 4(H)

Sequence of the H6-promoted human CEAmod/42K-promoted B7.1 insertion cassette (Cont.)

```
      5530       5540       5550       5560       5570       5580
TTATTTTTAT GTTTTAGTTA TTTGTTAGGT TATACAAAAA TTATGTTTAT TTGTGTATAT
AATAAAAATA CAAAATCAAT AAACAATCCA ATATGTTTTT AATACAAATA AACACATATA
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      5590       5600       5610       5620       5630       5640
TTAAAGCGTC GTTAAGAATA AGCTTAGTTA ACATATTATC GCTTAGGTTT TGTAGTATTT
AATTTCGCAG CAATTCTTAT TCGAATCAAT TGTATAATAG CGAATCCAAA ACATCATAAA
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      5650       5660       5670       5680       5690       5700
GAATCCTTTC TTTAAATGGA TTATTTTTCC AATGCATATT TATAGCTTCA TCCAAAGTAT
CTTAGGAAAG AAATTTACCT AATAAAAAGG TTACGTATAA ATATCGAAGT AGGTTTCATA
__________d___ALVAC'S C5 LOCUS RIGHT FLANKING ARM_____d__________>

      5710       5720       5730       5740       5750       5760
AACATTTAAC ATTCAGAATT GCGGCCGCAA TTCAATTCGT AATCATGGTC ATAGCTGTTT
TTGTAAATTG TAAGTCTTAA CGCCGGCGTT AAGTTAAGCA TTAGTACCAG TATCGACAAA
____ALVAC'S C5 LOCUS _d__>

      5770       5780       5790       5800       5810       5820
CCTGTGTGAA ATTGTTATCC GCTCACAATT CCACACAACA TACGAGCCGG AAGCATAAAG
GGACACACTT TAACAATAGG CGAGTGTTAA GGTGTGTTGT ATGCTCGGCC TTCGTATTTC

      5830       5840       5850       5860       5870       5880
TGTAAAGCCT GGGGTGCCTA ATGAGTGAGC TAACTCACAT TAATTGCGTT GCGCTCACTG
ACATTTCGGA CCCCACGGAT TACTCACTCG ATTGAGTGTA ATTAACGCAA CGCGAGTGAC

      5890       5900       5910       5920       5930       5940
CCCGCTTTCC AGTCGGGAAA CCTGTCGTGC CAGCTGCATT AATGAATCGG CCAACGCGCG
GGGCGAAAGG TCAGCCCTTT GGACAGCACG GTCGACGTAA TTACTTAGCC GGTTGCGCGC

      5950       5960       5970       5980       5990       6000
GGGAGAGGCG GTTTGCGTAT TGGGCGCTCT TCCGCTTCCT CGCTCACTGA CTCGCTGCGC
CCCTCTCCGC CAAACGCATA ACCCGCGAGA AGGCGAAGGA GCGAGTGACT GAGCGACGCG

      6010       6020       6030       6040       6050       6060
TCGGTCGTTC GGCTGCGGCG AGCGGTATCA GCTCACTCAA AGGCGGTAAT ACGGTTATCC
AGCCAGCAAG CCGACGCCGC TCGCCATAGT CGAGTGAGTT TCCGCCATTA TGCCAATAGG

      6070       6080       6090       6100       6110       6120
ACAGAATCAG GGGATAACGC AGGAAAGAAC ATGTGAGCAA AAGGCCAGCA AAAGGCCAGG
TGTCTTAGTC CCCTATTGCG TCCTTTCTTG TACACTCGTT TTCCGGTCGT TTTCCGGTCC

      6130       6140       6150       6160       6170       6180
AACCGTAAAA AGGCCGCGTT GCTGGCGTTT TTCCATAGGC TCCGCCCCCC TGACGAGCAT
TTGGCATTTT TCCGGCGCAA CGACCGCAAA AAGGTATCCG AGGCGGGGGG ACTGCTCGTA

      6190       6200       6210       6220       6230       6240
CACAAAAATC GACGCTCAAG TCAGAGGTGG CGAAACCCGA CAGGACTATA AAGATACCAG
GTGTTTTTAG CTGCGAGTTC AGTCTCCACC GCTTTGGGCT GTCCTGATAT TTCTATGGTC

      6250       6260       6270       6280       6290       6300
GCGTTTCCCC CTGGAAGCTC CCTCGTGCGC TCTCCTGTTC CGACCCTGCC GCTTACCGGA
CGCAAAGGGG GACCTTCGAG GGAGCACGCG AGAGGACAAG GCTGGGACGG CGAATGGCCT
```

Figure 4(I)

Sequence of the H6-promoted human CEAmod/42K-promoted B7.1 insertion cassette (Cont.)

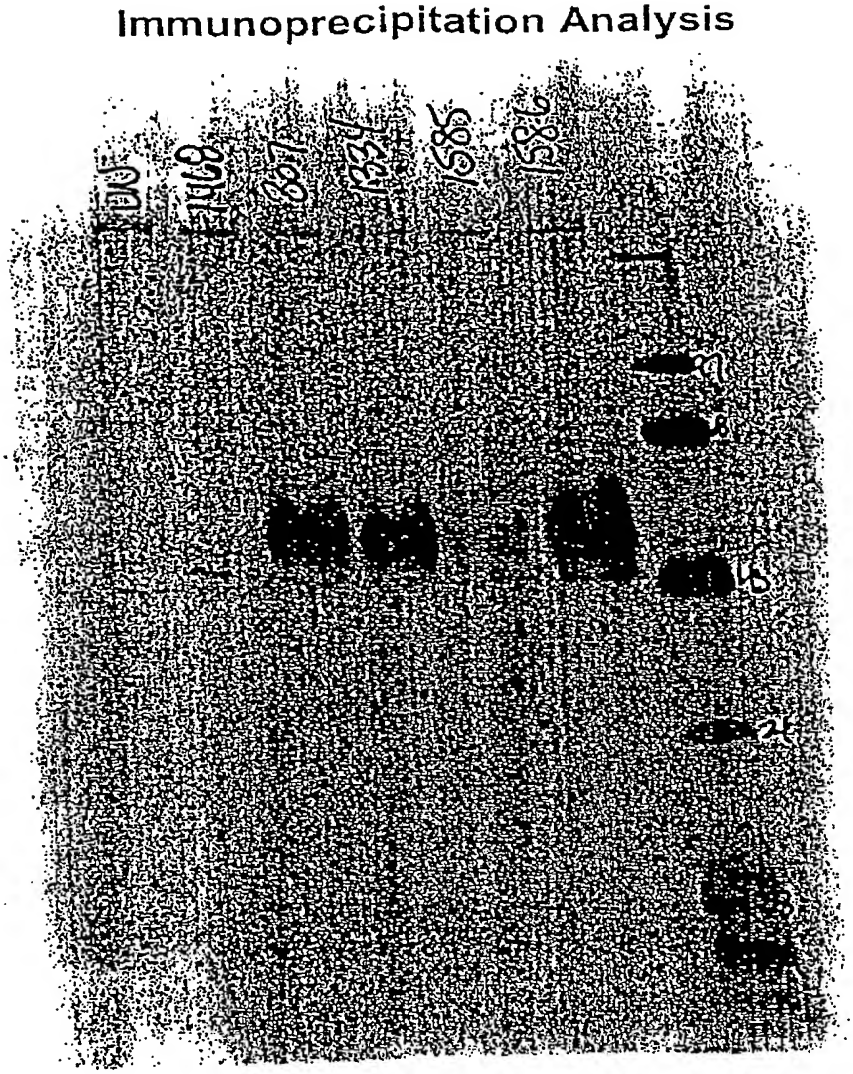
```
      6310        6320        6330        6340        6350        6360
TACCTGTCCG CCTTTCTCCC TTCGGGAAGC GTGGCGCTTT CTCATAGCTC ACGCTGTAGG
ATGGACAGGC GGAAAGAGGG AAGCCCTTCG CACCGCGAAA GAGTATCGAG TGCGACATCC

      6370        6380        6390        6400        6410        6420
TATCTCAGTT CGGTGTAGGT CGTTCGCTCC AAGCTGGGCT GTGTGCACGA ACCCCCCGTT
ATAGAGTCAA GCCACATCCA GCAAGCGAGG TTCGACCCGA CACACGTGCT TGGGGGGCAA

      6430        6440        6450        6460        6470        6480
CAGCCCGACC GCTGCGCCTT ATCCGGTAAC TATCGTCTTG AGTCCAACCC GGTAAGACAC
GTCGGGCTGG CGACGCGGAA TAGGCCATTG ATAGCAGAAC TCAGGTTGGG CCATTCTGTG

      6490        6500        6510        6520        6530        6540
GACTTATCGC CACTGGCAGC AGCCACTGGT AACAGGATTA GCAGAGCGAG GTATGTAGGC
CTGAATAGCG GTGACCGTCG TCGGTGACCA TTGTCCTAAT CGTCTCGCTC CATACATCCG

      6550        6560        6570        6580        6590        6600
GGTGCTACAG AGTTCTTGAA GTGGTGGCCT AACTACGGCT ACACTAGAAG GACAGTATTT
CCACGATGTC TCAAGAACTT CACCACCGGA TTGATGCCGA TGTGATCTTC CTGTCATAAA

      6610        6620        6630        6640        6650        6660
GGTATCTGCG CTCTGCTGAA GCCAGTTACC TTCGGAAAAA GAGTTGGTAG CTCTTGATCC
CCATAGACGC GAGACGACTT CGGTCAATGG AAGCCTTTTT CTCAACCATC GAGAACTAGG

      6670        6680        6690        6700        6710        6720
GGCAAACAAA CCACCGCTGG TAGCGGTGGT TTTTTTGTTT GCAAGCAGCA GATTACGCGC
CCGTTTGTTT GGTGGCGACC ATCGCCACCA AAAAAACAAA CGTTCGTCGT CTAATGCGCG

      6730        6740        6750        6760        6770        6780
AGAAAAAAAG GATCTCAAGA AGATCCTTTG ATCTTTTCTA CGGGGTCTGA CGCTCAGTGG
TCTTTTTTTC CTAGAGTTCT TCTAGGAAAC TAGAAAAGAT GCCCCAGACT GCGAGTCACC

      6790        6800        6810        6820        6830        6840
AACGAAAACT CACGTTAAGG GATTTTGGTC ATGAGATTAT CAAAAAGGAT CTTCACCTAG
TTGCTTTTGA GTGCAATTCC CTAAAACCAG TACTCTAATA GTTTTTCCTA GAAGTGGATC

      6850        6860        6870        6880        6890        6900
ATCCTTTTAA ATTAAAAATG AAGTTTTAAA TCAATCTAAA GTATATATGA GTAAACTTGG
TAGGAAAATT TAATTTTTAC TTCAAAATTT AGTTAGATTT CATATATACT CATTTGAACC

      6910        6920        6930        6940        6950        6960
TCTGACAGTT ACCAATGCTT AATCAGTGAG GCACCTATCT CAGCGATCTG TCTATTTCGT
AGACTGTCAA TGGTTACGAA TTAGTCACTC CGTGGATAGA GTCGCTAGAC AGATAAAGCA

      6970        6980        6990        7000        7010        7020
TCATCCATAG TTGCCTGACT CCCCGTCGTG TAGATAACTA CGATACGGGA GGGCTTACCA
AGTAGGTATC AACGGACTGA GGGGCAGCAC ATCTATTGAT GCTATGCCCT CCCGAATGGT

      7030        7040        7050        7060        7070        7080
TCTGGCCCCA GTGCTGCAAT GATACCGCGA GACCCACGCT CACCGGCTCC AGATTTATCA
AGACCGGGGT CACGACGTTA CTATGGCGCT CTGGGTGCGA GTGGCCGAGG TCTAAATAGT

      7090        7100        7110        7120        7130        7140
GCAATAAACC AGCCAGCCGG AAGGGCCGAG CGCAGAAGTG GTCCTGCAAC TTTATCCGCC
CGTTATTTGG TCGGTCGGCC TTCCCGGCTC GCGTCTTCAC CAGGACGTTG AAATAGGCGG

      7150        7160        7170        7180        7190        7200
```

Figure 4(J)

Sequence of the H6-promoted human CEAmod/42K-promoted B7.1 insertion cassette (Cont.)

```
TCCATCCAGT CTATTAATTG TTGCCGGGAA GCTAGAGTAA GTAGTTCGCC AGTTAATAGT
AGGTAGGTCA GATAATTAAC AACGGCCCTT CGATCTCATT CATCAAGCGG TCAATTATCA

      7210       7220       7230       7240       7250       7260
TTGCGCAACG TTGTTGCCAT TGCTACAGGC ATCGTGGTGT CACGCTCGTC GTTTGGTATG
AACGCGTTGC AACAACGGTA ACGATGTCCG TAGCACCACA GTGCGAGCAG CAAACCATAC

      7270       7280       7290       7300       7310       7320
GCTTCATTCA GCTCCGGTTC CCAACGATCA AGGCGAGTTA CATGATCCCC CATGTTGTGC
CGAAGTAAGT CGAGGCCAAG GGTTGCTAGT TCCGCTCAAT GTACTAGGGG GTACAACACG

      7330       7340       7350       7360       7370       7380
AAAAAAGCGG TTAGCTCCTT CGGTCCTCCG ATCGTTGTCA GAAGTAAGTT GGCCGCAGTG
TTTTTTCGCC AATCGAGGAA GCCAGGAGGC TAGCAACAGT CTTCATTCAA CCGGCGTCAC

      7390       7400       7410       7420       7430       7440
TTATCACTCA TGGTTATGGC AGCACTGCAT AATTCTCTTA CTGTCATGCC ATCCGTAAGA
AATAGTGAGT ACCAATACCG TCGTGACGTA TTAAGAGAAT GACAGTACGG TAGGCATTCT

      7450       7460       7470       7480       7490       7500
TGCTTTTCTG TGACTGGTGA GTACTCAACC AAGTCATTCT GAGAATAGTG TATGCGGCGA
ACGAAAAGAC ACTGACCACT CATGAGTTGG TTCAGTAAGA CTCTTATCAC ATACGCCGCT

      7510       7520       7530       7540       7550       7560
CCGAGTTGCT CTTGCCCGGC GTCAATACGG GATAATACCG CGCCACATAG CAGAACTTTA
GGCTCAACGA GAACGGGCCG CAGTTATGCC CTATTATGGC GCGGTGTATC GTCTTGAAAT

      7570       7580       7590       7600       7610       7620
AAAGTGCTCA TCATTGGAAA ACGTTCTTCG GGGCGAAAAC TCTCAAGGAT CTTACCGCTG
TTTCACGAGT AGTAACCTTT TGCAAGAAGC CCCGCTTTTG AGAGTTCCTA GAATGGCGAC

      7630       7640       7650       7660       7670       7680
TTGAGATCCA GTTCGATGTA ACCCACTCGT GCACCCAACT GATCTTCAGC ATCTTTTACT
AACTCTAGGT CAAGCTACAT TGGGTGAGCA CGTGGGTTGA CTAGAAGTCG TAGAAAATGA

      7690       7700       7710       7720       7730       7740
TTCACCAGCG TTTCTGGGTG AGCAAAAACA GGAAGGCAAA ATGCCGCAAA AAAGGGAATA
AAGTGGTCGC AAAGACCCAC TCGTTTTTGT CCTTCCGTTT TACGGCGTTT TTTCCCTTAT

      7750       7760       7770       7780       7790       7800
AGGGCGACAC GGAAATGTTG AATACTCATA CTCTTCCTTT TTCAATATTA TTGAAGCATT
TCCCGCTGTG CCTTTACAAC TTATGAGTAT GAGAAGGAAA AAGTTATAAT AACTTCGTAA

      7810       7820       7830       7840       7850       7860
TATCAGGGTT ATTGTCTCAT GAGCGGATAC ATATTTGAAT GTATTTAGAA AAATAAACAA
ATAGTCCCAA TAACAGAGTA CTCGCCTATG TATAAACTTA CATAAATCTT TTTATTTGTT

      7870       7880       7890       7900       7910       7920
ATAGGGGTTC CGCGCACATT TCCCCGAAAA GTGCCACCTG ACGTCTAAGA AACCATTATT
TATCCCCAAG GCGCGTGTAA AGGGGCTTTT CACGGTGGAC TGCAGATTCT TTGGTAATAA

      7930       7940       7950
ATCATGACAT TAACCTATAA AAATAGGCGT ATCACGAG
TAGTACTGTA ATTGGATATT TTTATCCGCA TAGTGCTC
```

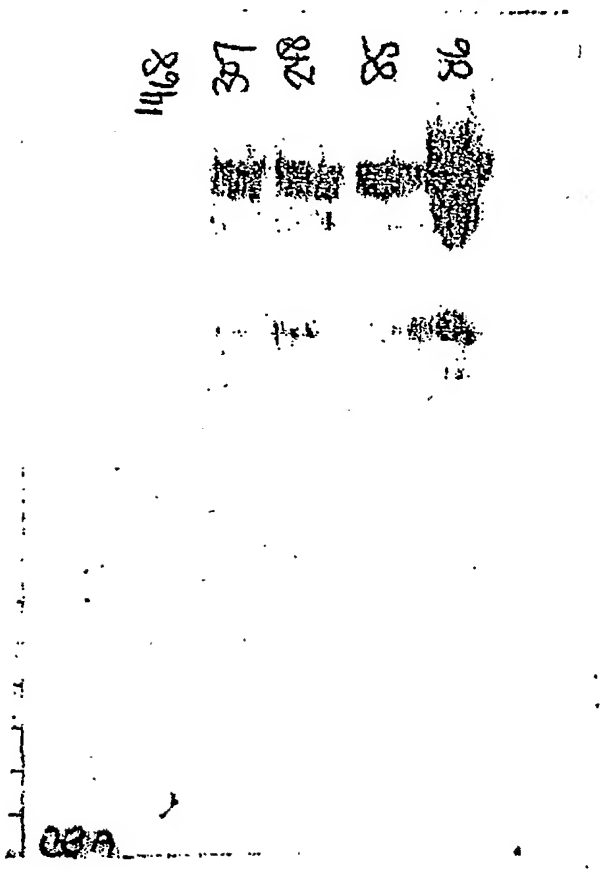
Figure 4(K)



Lane 1: Uninfected HeLA cells
Lane 2: HeLa cells infected with ALVAC(2) parental virus (vCP1468)
Lane 3: HeLa cells infected with ALVAC-CEA/B7.1 (vCP307)
Lane 4: HeLa cells infected with ALVAC-B7.1 (vCp1334)
Lane 5: HeLa cells infected with ALVAC-CEAmod/B7.1 (vCP1585)
Lane 6: HeLa cells infected with ALVAC(2)-CEAmod/B7.1 (vCP1586)
Molecular Weight Markers: 200, 98.6, 68, 43, 29, 18, 14 kDa

Figure 5

Western Blot Analysis



Lane 1: HeLa cells infected with ALVAC(2) parental virus (vCP1468)
Lane 2: HeLa cells infected with ALVAC-CEA/B7.1 (vCP307)
Lane 3: HeLa cells infected with ALVAC-CEA (vCP248)
Lane 4: HeLa cells infected with ALVAC-CEAmod/B7.1 (vCP1585)
Lane 6: HeLa cells infected with ALVAC(2)-CEAmod/B7.1 (vCP1586)

Figure 6

SEQUENCE LISTING

<110> AVENTIS PASTEUR LIMITED
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NATIONAL CANCER INSTITUTE
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<120> MODIFIED CEA AND USES THEREOF

<130> 1038-1144 LAB

<150> US 60/222,042
<151> 31/07/2000

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<170> PatentIn Ver. 2.0

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Thr Pro Phe Asn Val Ala Glu Gly Lys Glu Val Leu Leu Leu Val His Asn Leu Pro Gln
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His Leu Phe Gly Tyr Ser Trp Tyr Lys Gly Glu Arg Val Asp Gly Asn Arg Gln Ile Ile
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Gly Tyr Val Ile Gly Thr Gln Gln Ala Thr Pro Gly Pro Ala Tyr Ser Gly Arg Glu Ile
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Ile Tyr Pro Asn Ala Ser Leu Leu Ile Gln Asn Ile Ile Gln Asn Asp Thr Gly Phe Tyr
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Tyr Pro Glu Leu Pro Lys Pro Ser Ile Ser Ser Asn Asn Ser Lys Pro Val Glu Asp Lys
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Asp Ala Val Ala Phe Thr Cys Glu Pro Glu Thr Gln Asp Ala Thr Tyr Leu Trp Trp Val
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Thr Ile Ser Pro Leu Asn Thr Ser Tyr Arg Ser Gly Glu Asn Leu Asn Leu Ser Cys His
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